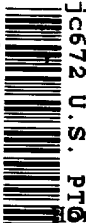


06/07/99



1c672 U.S. PT

Docket No. 59131/JPW/AKC/79

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Honorable Assistant Commissioner for Patents  
Washington, D.C. 20231

June 7, 1999

1c525 U.S. PT



S I R:

Transmitted herewith for filing are the specification and claims of the patent application of:

Taka-Aki Sato  
Inventor(s)GENE ENCODING NADE, p75<sup>NTR</sup> - ASSOCIATED CELL DEATH EXECUTOR AND USES THEREOF  
Title of Invention

Also enclosed are:

☒ 17 sheet(s) of ☐ informal ☒ formal drawings.☐ Oath or declaration of Applicant(s).☐ A power of attorney☐ An assignment of the invention to \_\_\_\_\_☒ A Preliminary Amendment☒ A verified statement to establish small entity status under 37 C.F.R. §1.9 and §1.27.

The filing fee is calculated as follows:

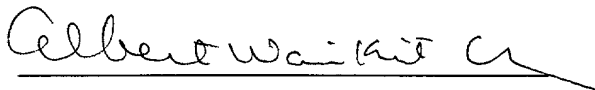
CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT

	NUMBER FILED		NUMBER EXTRA*		RATE		FEE	
					SMALL ENTITY	OTHER ENTITY	SMALL ENTITY	OTHER ENTITY
Total Claims	23 -20	=	3	X	\$ 9.00	\$18.00	= \$27.00	\$
Independent Claims	7 -3	=	1	X	\$39.00	\$78.00	= \$39.00	\$
Multiple Dependent Claims Presented: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No					\$130.00	\$260.00	= \$ 0	\$
*If the different in Col. 1 is less than zero, enter "0" in Col. 2					BASIC FEE		\$ 380	\$ 760
					TOTAL FEE		\$ 446.00	\$

Applicant: Taka-Aki Sato  
U.S. Serial No: Not Yet Known  
Filed: Herewith

- ☒ A check in the amount of \$ 446.00 to cover the filing fee.
- ☐ Please charge Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_.
- ☒ The Commissioner is hereby authorized to charge any additional fees which may be required in connection with the following or credit any over-payment to Account No. 03-3125 :
- ☐ Filing fees under 37 C.F.R. §1.16.
- ☐ Patent application processing fees under 37 C.F.R. §1.17.
- ☐ The issue fee set in 37 C.F.R. §1.18 at or before mailing of the Notice of Allowance, pursuant to 37 C.F.R. §1.311(b).
- ☒ Three copies of this sheet are enclosed.
- ☐ A certified copy of previously filed foreign application No. \_\_\_\_\_ filed in \_\_\_\_\_ on \_\_\_\_\_ . Applicant(s) hereby claim priority based upon this aforementioned foreign application under 35 U.S.C. §119.
- ☒ Other (identify) Express Mail Certificate of Mailing bearing label No. EJ 700 079 781 US and one loose set of figures

Respectfully submitted,



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Taka-Aki Sato  
Serial No. : Not Yet Known  
Filed : Herewith  
For : GENE ENCODING NADE, p75<sup>NTR</sup> - ASSOCIATED  
CELL DEATH EXECUTOR AND USES THEREOF

1185 Avenue of the Americas  
New York, New York 10036  
June 7, 1999

Assistant Commissioner for Patents  
Washington, D.C. 20231  
Box: Patent Application

Sir:

PRELIMINARY AMENDMENT

Please amend the above-identified application as follows:

In the claims:

Please cancel claims 5, 6, 7, 24-28, 30-54, 57-130 without prejudice to the applicants' rights to pursue the subject matters of these claims in a future application.

REMARKS

Claims 1-130 are pending in this application. Applicants have canceled claims 5, 6, 7, 24-28, 30-54, 57-130 without prejudice to applicants' the rights to pursue the subject matter of these claims in a future application. Upon entry of this amendment, claims 1-4, 8-23, 29, 55 and 56 are under examination. The Amendment does not contain issues of new matter and applicants respectfully request the entry of this Amendment.

Variable	Mean	SD	Min	Max
Age	35.2	10.5	18	65
Gender	Male	10.2	0	20
Marital status	Married	15.8	0	30
Education	High school	12.5	0	25
Occupation	Manager	18.3	0	35
Income	\$30,000	15.0	0	60
Health status	Good	10.0	0	20
Stress level	Low	12.0	0	25
Life satisfaction	High	15.0	0	30
Work-life balance	Good	18.0	0	35
Family support	High	20.0	0	40
Community involvement	Low	10.0	0	20
Volunteer work	Yes	15.0	0	30
Charitable contributions	Low	10.0	0	20
Political participation	Low	10.0	0	20
Civic engagement	Low	10.0	0	20
Neighborhood safety	High	15.0	0	30
Local government responsiveness	Low	10.0	0	20
Public services quality	High	15.0	0	30
Infrastructure development	Low	10.0	0	20
Environmental quality	High	15.0	0	30
Public transportation	Low	10.0	0	20
Healthcare access	High	15.0	0	30
Educational opportunities	Low	10.0	0	20
Job market stability	High	15.0	0	30
Economic growth	Low	10.0	0	20
Government transparency	High	15.0	0	30
Corruption levels	Low	10.0	0	20
Legal system efficiency	High	15.0	0	30
Media freedom	Low	10.0	0	20
Human rights protection	High	15.0	0	30
International relations	Low	10.0	0	20
Global peace	High	15.0	0	30
Climate change impact	Low	10.0	0	20
Renewable energy adoption	High	15.0	0	30
Technological innovation	Low	10.0	0	20
Digital literacy	High	15.0	0	30
Internet access	Low	10.0	0	20
Mobile phone usage	High	15.0	0	30
Smartphone ownership	Low	10.0	0	20
Online shopping	High	15.0	0	30
E-commerce growth	Low	10.0	0	20
Digital marketing	High	15.0	0	30
Social media usage	Low	10.0	0	20
Online news consumption	High	15.0	0	30
Digital privacy	Low	10.0	0	20
Data security	High	15.0	0	30
Cybersecurity awareness	Low	10.0	0	20
Digital citizenship	High	15.0	0	30
Online community participation	Low	10.0	0	20
Digital activism	High	15.0	0	30
Online petition signing	Low	10.0	0	20
Digital advocacy	High	15.0	0	30
Online fundraising	Low	10.0	0	20
Digital philanthropy	High	15.0	0	30
Online volunteering	Low	10.0	0	20
Digital social responsibility	High	15.0	0	30
Online corporate social responsibility	Low	10.0	0	20
Digital consumerism	High	15.0	0	30
Online consumer behavior	Low	10.0	0	20
Digital marketing research	High	15.0	0	30
Online brand loyalty	Low	10.0	0	20
Digital customer service	High	15.0	0	30
Online customer feedback	Low	10.0	0	20
Digital product development	High	15.0	0	30
Online user experience	Low	10.0	0	20
Digital innovation	High	15.0	0	30
Online entrepreneurship	Low	10.0	0	20
Digital business growth	High	15.0	0	30
Online business model innovation	Low	10.0	0	20
Digital market research	High	15.0	0	30
Online competitive analysis	Low	10.0	0	20
Digital strategic planning	High	15.0	0	30
Online business development	Low	10.0	0	20
Digital sales and marketing	High	15.0	0	30
Online sales funnel optimization	Low	10.0	0	20
Digital customer acquisition	High	15.0	0	30
Online lead generation	Low	10.0	0	20
Digital brand management	High	15.0	0	30
Online brand positioning	Low	10.0	0	20
Digital brand identity	High	15.0	0	30
Online brand consistency	Low	10.0	0	20
Digital brand loyalty	High	15.0	0	30
Online brand advocacy	Low	10.0	0	20
Digital brand reputation	High	15.0</		

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Applicant or Patentee: Taka-Aki Sato Attorney's  
Serial or Patent No.: Not Yet Known Docket No: 59131/JPW/WL  
Filed or Issued: Herewith  
Title of Invention or Patent: GENE ENCODING NADE.p75<sup>NTR</sup> - ASSOCIATED CELL DEATH  
EXECUTOR AND USES THEREOF

VERIFIED STATEMENT (DECLARATION) CLAIMING  
SMALL ENTITY STATUS UNDER 37 C.F.R. §1.9(f)  
AND §1.27(d) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: The Trustees of Columbia University in the City of New York

Address of Organization: 411 Low Memorial Library, West 116th Street and Broadway  
New York, New York 10027

TYPE OF ORGANIZATION:

☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION  
☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE 26 U.S.C. §§501(a) and 501(c)(3)  
☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA  
NAME OF STATE: \_\_\_\_\_  
CITATION OF STATUTE: \_\_\_\_\_  
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE 26 U.S.C. §§501(a) and 501(c)(3) IF LOCATED IN THE UNITED STATES OF AMERICA  
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA  
NAME OF STATE: \_\_\_\_\_  
CITATION OF STATUTE: \_\_\_\_\_

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. §1.9(e)\* for purposes of paying reduced fees under 35 U.S.C. §41(a) and 41(b), with regard to the invention entitled  
GENE ENCODING NADE.p75<sup>NTR</sup> - ASSOCIATED CELL DEATH EXECUTOR AND USES THEREOF

by inventor(s) Taka-Aki Sato

described in:

☒ the specification filed herewith  
☐ application serial no. \_\_\_\_\_ filed \_\_\_\_\_  
☐ patent no. \_\_\_\_\_ issued \_\_\_\_\_

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive each individual, concern, or organization known to have rights to the invention is listed below<sup>a</sup> and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. §1.9(d)\* or a nonprofit organization under 37 C.F.R. §1.9(e)\*

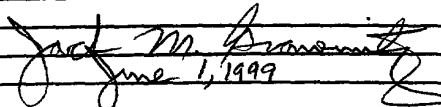
<sup>a</sup>NOTE. Separate verified statements are required from each person, concern, or organization having rights to the invention averring to their status as small entities. 37 C.F.R. §1.27.

Name: \_\_\_\_\_  
Address: \_\_\_\_\_

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. 37 C.F.R. §1.28(b)\*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Mr. Jack M. Granowitz  
Title In Organization: Executive Director, Columbia Innovation Enterprise  
Address: Amsterdam & 120th Street - Suite 363 New York, New York 10027  
Signature:   
Date Of Signature: June 1, 1999

Application  
for  
United States Letters Patent

003345-000709

To all whom it may concern:

Be it known that

I, Taka-Aki Sato  
have invented certain new and useful improvements in

Gene Encoding NADE, p75<sup>NTR</sup>-Associated Cell Death  
Executor and Uses Thereof

of which the following is a full, clear and exact description.

GENE ENCODING NADE, P75<sup>NTR</sup>-ASSOCIATED CELL DEATH  
EXECUTOR AND USES THEREOF

5      This invention described herein was supported by  
National Institutes of Health grant R01-GM55147.  
Accordingly, the United States Government has certain  
rights in this invention.

10     Throughout this application various publications are  
referred to within parenthesis. Full citations for  
these publications may be found at the end of the  
specification immediately preceding the claims. The  
disclosures of these publications, in their entireties,  
15     are hereby incorporated by reference into this  
application in order to more fully describe the state  
of the art to which this invention pertains.

Background of the Invention

20     The low-affinity neurotrophin receptor (p75<sup>NTR</sup>) can  
mediate cell survival or cell death by NGF or another  
neurotrophins stimulation in neuronal cells (1, 2, 3).  
To elucidate p75<sup>NTR</sup>-mediated signal transduction, the  
yeast two-hybrid system was employed to screen the  
25     mouse embryo cDNA libraries using the rat p75<sup>NTR</sup>ICD  
(intracellular domain) as a target. One positive clone  
was identified and termed NADE (p75<sup>NTR</sup>-associated cell  
death executor). NADE has a significant homology to  
30     human HGR74 protein (4) and does not have a typical  
biochemical motif except the consensus sequences of  
nuclear export signal (NES) (5) and ubiquitination (6).

Expression of NADE mRNA was found highest in brain,  
heart, and lung. NADE specifically binds to p75<sup>NTR</sup>ICD  
35     both *in vitro* and *in vivo*. Co-expression of NADE  
together with p75<sup>NTR</sup> dramatically induced Caspase-2 and  
Caspase-3 activities to cleave PARP (poly (ADP-ribose)  
polymerase) and fragmentation of nuclear DNA in 293T  
cells, but NADE without p75<sup>NTR</sup> did not show apoptosis,  
40     suggesting that NADE expression is necessary for p75<sup>NTR</sup>-



5 Interestingly, NADE protein is found to be ubiquitinated as a substrate for protein degradation pathway. Taken together, NADE is the first signal adaptor molecule identified in involvement of p75<sup>NTR</sup>-mediated apoptosis, and it may play an important role  
10 in the pathogenesis of neurogenetic diseases.

Summary of the Invention

5 This invention provides an isolated nucleic molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor.

10 This invention provides a method of producing a polypeptide capable of binding a p75<sup>NTR</sup> receptor which comprises growing host cells selected from a group consisting of bacterial, plant, insect or mammalian cell, under suitable conditions permitting production of the polypeptide.

15 This invention provides an antisense oligonucleotide having a nucleic acid sequence capable of specifically hybridizing to an mRNA molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor.

20 This invention provides a purified polypeptide capable of binding a p75<sup>NTR</sup> receptor.

25 This invention provides a method of producing a polypeptide capable of binding a p75<sup>NTR</sup> receptor into a suitable vector which comprises: (a) inserting a nucleic acid molecule encoding the polypeptide capable of binding a p75<sup>NTR</sup> receptor into a suitable vector; (b) introducing the resulting vector into a suitable host cell; (c) selecting the introduced host cell for the expression of the polypeptide capable of binding  
30 a p75<sup>NTR</sup> receptor; (d) culturing the selected cell to produce the polypeptide capable of binding a p75<sup>NTR</sup> receptor; and (e) recovering the polypeptide capable of binding a p75<sup>NTR</sup> receptor produced.

35 This invention provides a method of identifying a compound capable of inhibiting binding between p75<sup>NTR</sup>

receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor, where said binding forms a complex between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor, comprising: a) contacting the compound  
5 under conditions permitting the binding of the polypeptide capable of binding p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> receptor with the polypeptide capable of binding p75<sup>NTR</sup> receptor to form a mixture; b) contacting p75<sup>NTR</sup> receptor with the mixture from step a); and  
10 c) measuring the amount of complexed p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor.

This invention provides a method of identifying a compound capable of inhibiting binding between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor, where said binding forms a complex between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor, comprising: a) contacting the compound under conditions permitting the binding of  
15 the polypeptide capable of binding p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> receptor with the p75<sup>NTR</sup> receptor to form a mixture; b) contacting the polypeptide capable of binding a p75<sup>NTR</sup> receptor with the mixture from step a); and c) measuring the amount of complexed p75<sup>NTR</sup> receptor and a polypeptide.  
25

This invention provides a method of inducing apoptosis in cells which comprises expressing a polypeptide capable of binding a p75<sup>NTR</sup> receptor in the cells.  
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This invention provides a method of inducing apoptosis in a subject which comprises expressing a polypeptide capable of binding a p75<sup>NTR</sup> receptor in the subject.

35 This invention provides a method of determining physiological effects of expressing varying levels of a polypeptide capable of binding a p75<sup>NTR</sup> receptor in

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This invention provides a method to inhibit NF- $\kappa$ B activation in a cell with human HGR74 protein and p75<sup>NTR</sup>.

5 This invention provides a method to detect a neurodegenerative disease in a subject by detecting expression levels of a polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>.

10 This invention provides a method of identifying a compound, which is an apoptosis inhibitor, said compound is capable of inhibiting specific binding between a polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> receptor, so as to prevent  
15 apoptosis which comprises: (a) contacting the polypeptide capable of binding a p75<sup>NTR</sup> receptor with a plurality of compounds under conditions permitting binding between a known compound previously shown to be able to displace the polypeptide capable of binding  
20 a p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor and the bound p75<sup>NTR</sup> receptor to form a complex; and (b) detecting the displaced polypeptide capable of binding a p75<sup>NTR</sup> receptor or the complex formed in step (a), wherein the displacement indicates that the compound is  
25 capable of inhibiting specific binding between the polypeptide capable of binding a p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor.

30 This invention provides a method of identifying a compound, which is an apoptosis inhibitor, said compound is capable of inhibiting specific binding between human HGR74 protein and p75<sup>NTR</sup> receptor, so as to prevent apoptosis which comprises: (a) contacting the human HGR74 protein with a plurality of compounds  
35 under conditions permitting binding between a known compound previously shown to be able to displace the human HGR74 protein and the p75<sup>NTR</sup> receptor and the

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## 5

C=cytosine  
A=adenosine  
T=thymidine  
G=guanosine

As used herein, amino acid residues are abbreviated as follows:

A=Alanine  
C=Cysteine  
D=Aspartic Acid  
E=Glutamic Acid  
F=Phenylalanine  
G=Glycine

H=Histidine  
I=Isoleucine  
K=Lysine  
L=Leucine  
M=Methionine

N=Asparagine  
P=Proline  
Q=Glutamine  
R=Arginine  
S=Serine

T=Threonine  
V=Valine  
W=Tryptophan  
Y=Tyrosine

## 35 Figure legends

**Fig. 1 A-H** Amino acid sequence and expression analysis

of NADE.

**Figure 1A**

Amino acid alignment of mouse and human NADE (HGR74)  
5 (4) proteins. The dotted sequence is asparagine rich  
stretch. The asterisks indicate the leucine-rich  
nuclear export signal (NES)(5). The closed triangle  
indicates cysteine residue essential for dimmer  
formation. The prenylation sequence in C-termini is  
10 underlined.

**Figure 1B**

Comparison of leucine-rich nuclear export signal (NES)  
(5) in various protein. The consensus sequence for  
15 NES are shadowed. Genbank accession numbers are:  
cZyxin, X69190; MAPKK, D13700; PKI-a, L02615; TFIIIA,  
M85211; RevHIV-1, AF075719; RanBP1, L25255; FMRP,  
L29074; Gle1, U68475; Human NADE, submitted; mouse  
NADE, submitted.

20

**Figure 1C**

Consensus sequence of ubiquitination signal.

**Figure 1D**

25 Northern blot analysis of NADE.

**Figure 1E**

Expression of endogenous NADE protein in SK-N-MC human  
neuroblastoma cells. SK-N-MC cell lysate treated with  
30 ALLN is immunoprecipitated by anti-NADE antibody, and  
subjected to immunoblotting by same antibody. Human  
NADE protein transiently expressed in 293T cells and  
untreated gels were used for controls. Heavy chain  
bands are resulted from antibodies using  
35 immunoprecipitation.

**Figure 1F**

Mutant analysis of mouse NADE protein A wild type NADE, muNADE(Cys102Ser), and muNADE(Cys121Ser) proteins transiently expressed in 293T cells were detected by immunoblotting with anti-NADE antibody.

5 Transfection methods are described in material and methods. The cell lysate extracted from the 293T cells transfected with parental vector was used as a control.

10 **Figure 1G-1 and 1G-2**

Blast Search and comparison of mouse NADE nucleic acid sequence Figure 1G-1 (SEQ ID NO: \_\_) and human protein HGR74 sequence

15 **Figure 1H**

Comparison of mouse NADE, human HGR74 protein and other homologous rat, mouse and human amino acid sequences

20 **Fig. 2A-C** NADE binds to p75<sup>NTR</sup> strongly *in vitro* and *in vivo*.

**Figure 2A**

*In vitro* binding assay of NADE and p75<sup>NTR</sup>. *In vitro*-translated NADE protein was subjected to GST-pull down assay using a GST-p75<sup>NTR</sup>ICD fusion protein. GST was used as a control.

**Figure 2B**

30 *In vivo* binding assay of NADE and p75<sup>NTR</sup>. The cell lysates extracted from 293T cells co-transfected with Myc-tagged NADE and p75<sup>NTR</sup> were co-immunoprecipitated by anti-Myc antibody, and subjected to immunoblotting by anti-p75<sup>NTR</sup> antibody. The lysates from the cells  
35 transfected with each plasmid and a parental vector were used as controls. Transfection methods are described in material and methods.

**Figure 2C**

Interaction of NADE with p75<sup>NTR</sup> depending on NGF ligation. 293T cells co-transfected with Myc-tagged NADE and p75<sup>NTR</sup> were treated with NGF in various concentration as indicated. Upper panel; Immunoprecipitates of anti-Myc antibody (IgG1) from each sample were subjected to immunoblotting analysis by anti p75<sup>NTR</sup> antibody. Middle and lower panels indicated the expression level of p75<sup>NTR</sup> and NADE proteins by immunoblotting, respectively. The immunoprecipitate of anti-FLAG antibody (IgG1) was used as a control.

**Fig. 3A-E** Effect of NADE and p75<sup>NTR</sup> co-transfection on 293T cells.

**Figure 3A**

Morphological change caused by co-transfection of NADE and p75<sup>NTR</sup> in 293T cells transfected by each cDNA were observed 48 hours after transfection. The magnification was 200. Transfection methods are described in material and methods.

**Figure 3B**

TUNEL assay. Transfected 293T cells were stained by  
TUNEL method and analyzed by a flow cytometer. The  
5 percentages indicated are positive populations.

**Figure 3C**

DNA fragmentation analysis. DNAs from transfected  
293T cells were checked by 1.5 % agarose gel  
10 electrophoresis.

**Figure 3D**

Inhibition of NF- $\kappa$ B activity by NADE. NF- $\kappa$ B activities  
in transfected cells were measured by E-selectin  
15 promoter-luciferase gene reporter assay. Luciferase  
activities were determined 24 hours after transfection  
and normalized on the basis of pRL-TK expression  
levels.

**Figure 3E**

Activation of Caspase-2 and 3 and degradation of PARP  
in co-transfected 293T cells. The cell extracts from  
293T cells transfected by each cDNA as indicated were  
analyzed by immunoblotting with anti-Caspase-2,  
25 Caspase-3, and PAPP antibody. The level of  $\alpha$ -tubulin  
was measured as a control.

**Fig. 4A-D** A conserved Rev-like NES in the C-terminus  
mediates nuclear export of NADE protein.

30

**Figure 4A**

At residues 88-100, the mouse NADE NES lies within the  
C-terminus. A mouse NADE is aligned with homologous  
sequences of NADE family members and the NES sequences  
35 of HIV Rev, MAPKK, cZyxin and PKI-a.

**Figure 4B**

Subcellular localization of a wild type mNADE-GFP and a control GFP vector was analyzed in transfected 293T cells.

5     **Figure 4C**

Effects of deletion mutants of NES motif on nuclear export of GFP-fused mouse NADE proteins. Both deletion mutants with or without NES indicate deletion-124 and delta 91-124, respectively.

10

**Figure 4D**

Effects of point mutations within the NES motif on nuclear export of GFP-fused mouse NADE proteins. The single or double amino acid substitutions were made at residue 94 and 97 (Leu to Ala). GFP-constructs were transiently transfected into 293T cells. The fixed cells were stained with TO-PRO-3 to visualize the nucleus and images of representative cell fields were captured on a confocal laser microscope. More than 1000 cells were analyzed for each construct.

15

20

**Detailed Description of the Invention**

The following standard abbreviations are used throughout the specification to indicate specific nucleotides:

C=cytosine  
A=adenosine  
T=thymidine  
G=guanosine

As used herein, amino acid residues are abbreviated as follows:

A=Alanine  
C=Cysteine  
D=Aspartic Acid  
E=Glutamic Acid  
F=Phenylalanine  
G=Glycine  
H=Histidine  
I=Isoleucine  
K=Lysine  
L=Leucine  
M=Methionine  
N=Asparagine  
P=Proline  
Q=Glutamine  
R=Arginine  
S=Serine  
T=Threonine  
V=Valine  
W=Tryptophan  
Y=Tyrosine

This invention provides an isolated nucleic molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup>

receptor. In an embodiment of the above described isolated nucleic molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor the isolated nucleic acid is a DNA molecule. In another embodiment of the above described isolated nucleic acid molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor the isolated nucleic acid is a cDNA molecule. In a further embodiment of the above described isolated DNA molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor the isolated nucleic acid is a RNA molecule. In an embodiment of the above described isolated nucleic acid molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor, the isolated nucleic acid is operatively linked to a promoter of RNA transcription. In yet another embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule encodes a neurotrophin associated cell death executor protein. In an embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule comprises a sequence of AATTG TCTAC GCATC CTTAT GGGGG AGCTG TCTAA C.

As used herein, "polypeptide" includes both peptides and proteins. "Peptide" means a polypeptide of fewer than 10 amino acid residues in length, and "protein" means a polypeptide of 10 or more amino acid residues in length. In this invention, the polypeptides may be naturally occurring or recombinant (i.e. produced via recombinant DNA technology), and may contain mutations (e.g. point, insertion and deletion mutations) as well as other covalent modifications (e.g. glycosylation and labeling [via biotin, streptavidin, fluoracine, and radioisotopes such as <sup>131</sup>I]). Moreover, each instant composition may contain more than a single



polypeptide, i.e., each may be a monomer (one polypeptide bound to a polymer) or a multimer (two or more polypeptides bound to a polymer or to each other).

5

The p75<sup>NTR</sup> receptor is a low affinity nerve growth factor (NGF) receptor with a low affinity to neurotrophins. p75<sup>NTR</sup> receptor has been implicated in the mediation of cell death and cell survival.

10

"Capable of binding" is defined as the ability of a protein or other peptide molecule capable of recognizing and interacting with a complementary receptor site, which can be another protein or other type of molecule.

15

The DNA molecules of the subject invention also include DNA molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

35

662090-032660

5 The DNA molecules described and claimed herein are  
useful for the information which they provide  
concerning the amino acid sequence of the polypeptide  
capable of binding a p75<sup>NTR</sup> receptor, and as products  
for the large scale synthesis of the polypeptide  
capable of binding a p75<sup>NTR</sup> receptor, or fragments  
thereof, by a variety of recombinant techniques. The  
DNA molecule is useful for generating new cloning and  
10 expression vectors, transformed and transfected  
prokaryotic and eukaryotic host cells, and new and  
useful methods for cultured growth of such host cells  
capable of expression of the polypeptide capable of  
binding a p75<sup>NTR</sup> receptor or portions thereof and  
15 related products.

20 This invention provides a vector which comprises the  
isolated nucleic acid encoding a polypeptide capable  
of binding a p75<sup>NTR</sup> receptor, operatively linked to a  
promoter of RNA transcription. In an embodiment of  
the invention, where in the vector which comprises the  
isolated nucleic acid encoding a polypeptide capable  
of binding a p75<sup>NTR</sup> receptor, operatively linked to a  
promoter of RNA transcription is a plasmid. In  
25 another embodiment the above described isolated  
nucleic acid molecule which is a cDNA molecule  
encoding a polypeptide capable of binding a p75<sup>NTR</sup>  
receptor, encodes a human or mouse protein. In yet  
another embodiment the above described isolated  
30 nucleic acid molecule is a cDNA molecule wherein the  
nucleic acid molecule encodes a polypeptide capable of  
binding a p75<sup>NTR</sup> receptor comprising the amino acid  
sequence set forth in Figure 1G-1 (SEQ ID NO: \_\_\_\_). In  
a further embodiment the above described isolated  
35 nucleic acid molecule is a cDNA molecule wherein the

nucleic acid molecule encodes a polypeptide capable of binding a p75<sup>NTR</sup> receptor. In an embodiment of the above described isolated nucleic acid molecule which is a cDNA molecule wherein the nucleic acid molecule  
5 encodes a polypeptide capable of binding a p75<sup>NTR</sup> receptor which is a mouse, rat or human protein. In yet another embodiment of the above described isolated nucleic acid molecule which is a cDNA molecule, said isolated nucleic acid molecule comprises the nucleic  
10 acid sequence set forth in Figure 1G-1 (SEQ ID NO: \_\_\_\_).

Numerous vectors for expressing the inventive proteins may be employed. Such vectors, including plasmid  
15 vectors, cosmid vectors, bacteriophage vectors and other viruses, are well known in the art. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus,  
20 baculovirus, retroviruses (RSV, MMTV or MoMLV), Semliki Forest virus or SV40 virus. Additionally, cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow for the selection of transfected  
25 host cells. The markers may provide, for example, prototrophy to an auxotrophic host, biocide resistance or resistance to heavy metals such as copper. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced  
30 into the same cell by cotransformation.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome  
35 binding. Additional elements may also be needed for

optimal synthesis of mRNA. These additional elements may include splice signals, as well as enhancers and termination signals. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general.

These vectors may be introduced into a suitable host cell to form a host vector system for producing the inventive proteins. Methods of making host vector systems are well known to those skilled in the art.

Methods of introducing nucleic acid molecules into cells are well known to those of skill in the art. Such methods include, for example, the use of viral vectors and calcium phosphate co-precipitation.

This invention provides a host cell comprising the vector comprising the nucleic acid molecule of encoding a polypeptide capable of binding p75<sup>NTR</sup> receptor. In an embodiment the above described host cell is selected from a group consisting of a bacterial cell, a plant cell, and insect cell, and a mammalian cell.

The "suitable host cell" in which the nucleic acid molecule encoding is a polypeptide capable of binding

a p75<sup>NTR</sup> receptor capable of being expressed is any cell capable of taking up the nucleic acid molecule and stably expressing the polypeptide capable of binding a p75<sup>NTR</sup> receptor encoded thereby.

5

Suitable host cells include, but are not limited to, bacterial cells (including gram positive cells), yeast cells, fungal cells, insect cells and animal cells. Suitable animal cells include, but are not limited to HeLa cells, Cos cells, CV1 cells and various primary mammalian cells. Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH-3T3 cells, CHO cells, HeLa cells, Ltk<sup>-</sup> cells and COS cells. Mammalian cells may be transfected by methods well known in the art such as calcium phosphate precipitation, electroporation and microinjection.

This invention provides a method of producing a polypeptide having the biological activity of a polypeptide capable of binding a p75<sup>NTR</sup> receptor which comprises growing host cells selected from a group consisting of bacterial, plant, insect or mammalian cell, under suitable conditions permitting production of the polypeptide. In another embodiment of the above described method of producing a polypeptide having the biological activity of a polypeptide capable of binding a p75<sup>NTR</sup> receptor, the method further comprises the recovering of the produced polypeptide.

This invention provides an isolated nucleic acid molecule of at least 15 contiguous nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of the nucleic acid

5 molecule encoding a polypeptide capable of binding a  
p75<sup>NTR</sup> receptor. In an embodiment of the above  
described isolated nucleic acid molecule of at least  
15 contiguous nucleotides capable of specifically  
hybridizing with a unique sequence included within the  
sequence of the nucleic acid molecule encoding a  
polypeptide capable of binding a p75<sup>NTR</sup> receptor, said  
isolated nucleic acid molecule is a DNA molecule. In  
another embodiment of the above described isolated  
10 nucleic acid molecule of at least 15 contiguous  
nucleotides capable of specifically hybridizing with  
a unique sequence included within the sequence of the  
nucleic acid molecule encoding a polypeptide capable  
of binding a p75<sup>NTR</sup> receptor, said isolated nucleic  
15 molecule is a RNA molecule.

20 This invention provides an isolated nucleic acid  
molecule capable of specifically hybridizing with a  
unique sequence included within the sequence of a  
nucleic acid molecule which is complementary to the  
nucleic acid molecule encoding a polypeptide capable  
of binding a p75<sup>NTR</sup> receptor. In an embodiment the  
above described isolated nucleic acid molecule which  
is complementary to the nucleic acid molecule encoding  
25 a polypeptide capable of binding a p75<sup>NTR</sup> receptor is  
a DNA molecule. In another embodiment the above  
described isolated nucleic acid molecule capable of  
specifically hybridizing with a nucleic acid molecule  
capable of specifically hybridizing with a unique  
30 sequence included within the sequence of a nucleic  
acid molecule which is complementary to the nucleic  
acid molecule encoding a polypeptide capable of  
binding a p75<sup>NTR</sup> receptor is a RNA molecule.

35 One of ordinary skill in the art will easily obtain

unique sequences from the cDNA cloned in the polypeptide capable of binding a p75<sup>NTR</sup> receptor plasmid. Such unique sequences may be used as probes to screen various mammalian cDNA libraries and genomic DNAs, e.g. mouse, rat and bovine, to obtain homologous nucleic acid sequences and to screen different cDNA tissue libraries to obtain isoforms of the obtained nucleic acid sequences. Nucleic acid probes from the cDNA cloned in the polypeptide capable of binding a p75<sup>NTR</sup> receptor plasmid may further be used to screen other human tissue cDNA libraries to obtain isoforms of the nucleic acid sequences encoding polypeptide capable of binding a p75<sup>NTR</sup> receptor as well as to screen human genomic DNA to obtain the analogous nucleic acid sequences. The homologous nucleic acid sequences and isoforms may be used to produce the proteins encoded thereby.

As used herein, "capable of specifically hybridizing" means capable of binding to an mRNA molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor but not capable of binding to a polypeptide capable of binding a p75<sup>NTR</sup> receptor molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor.

This invention provides an antisense oligonucleotide having a nucleic acid sequence capable of specifically hybridizing to an mRNA molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor. In an embodiment of the above described antisense oligonucleotide, said antisense oligonucleotide has a nucleic acid sequence capable of specifically hybridizing to the isolated cDNA molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor. In another embodiment of the above described antisense oligonucleotide has a

nucleic acid sequence capable of specifically hybridizing to the isolated RNA molecule encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor.

5 This invention provides a purified a polypeptide capable of binding a p75<sup>NTR</sup> receptor. In an embodiment of the above described purified polypeptide capable of binding p75<sup>NTR</sup> receptor is encoded by the isolated nucleic acid encoding a polypeptide capable of binding  
10 a p75<sup>NTR</sup> receptor. In an embodiment the above described polypeptide capable of binding a p75<sup>NTR</sup> receptor is a fragment of the purified polypeptide capable of binding a p75<sup>NTR</sup> receptor. In another embodiment the above described purified polypeptide  
15 capable of binding a p75<sup>NTR</sup> receptor has substantially the same amino acid sequence as set forth in Figure 1G-1 (SEQ ID NO: \_\_). In a further embodiment the above described purified polypeptide capable of binding a p75<sup>NTR</sup> receptor having an amino acid sequence  
20 as set forth in Figure 1G-1 (SEQ ID NO: \_\_). In yet another embodiment the above described polypeptide capable of binding a p75<sup>NTR</sup> receptor has an amino acid sequence as set forth in Figure 1G-1 (SEQ ID NO: \_\_). In a further embodiment, the above described  
25 polypeptide capable of binding a p75<sup>NTR</sup> receptor is a vertebrate polypeptide capable of binding a p75<sup>NTR</sup> receptor. In an embodiment of the above described polypeptide capable of binding a p75<sup>NTR</sup> receptor comprises a neurotrophin associated cell death  
30 executor protein. In yet another embodiment of the above described polypeptide capable of binding a p75<sup>NTR</sup> receptor comprises NCLRILMGELSN.

As used herein, purified polypeptides means the  
35 polypeptides free of any other polypeptides.



As used herein, a polypeptide capable of binding a p75<sup>NTR</sup> receptor having "substantially the same" amino acid sequences as set forth in Figure 1G-1 (SEQ ID NO: \_\_) is encoded by a nucleic acid encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor, said nucleic acid having 100% identity in the homeodomain regions, that is those regions coding the protein, and said nucleic acid may vary in the nucleotides in the non-coding regions.

This invention provides a monoclonal antibody directed to an epitope of a polypeptide capable of binding a p75<sup>NTR</sup> receptor. In an embodiment the above described monoclonal antibody, said monoclonal antibody is directed to a mouse, rat or human polypeptide capable of binding a p75<sup>NTR</sup> receptor.

The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies. Specifically, the term "antibody" includes polyclonal and monoclonal antibodies, and fragments thereof. Furthermore, the term "antibody" includes chimeric antibodies, wholly synthetic antibodies, and fragments thereof. Optionally, an antibody can be labeled with a detectable marker. Detectable markers include, for example, radioactive or fluorescent markers.

This invention provides a polyclonal antibody directed to an epitope of the purified protein having the amino sequence as set forth in Figure 1G-1 (SEQ ID NO: \_\_). In a further embodiment the above described monoclonal or polyclonal antibodies are directed to the polypeptide capable of binding a p75<sup>NTR</sup> receptor, having the amino sequence as set forth in Figure 1G-1

(SEQ ID NO: \_\_).

Polyclonal antibodies may be produced by injecting a host animal such as rabbit, rat, goat, mouse or other animal with the immunogen of this invention, e.g. a purified mammalian polypeptide capable of binding a p75<sup>NTR</sup> receptor or a purified human polypeptide capable of binding a p75<sup>NTR</sup> receptor. The sera are extracted from the host animal and are screened to obtain polyclonal antibodies which are specific to the immunogen. Methods of screening for polyclonal antibodies are well known to those of ordinary skill in the art such as those disclosed in Harlow & Lane, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratories, Cold Spring Harbor, NY: 1988) the contents of which are hereby incorporated by reference.

The monoclonal antibodies may be produced by immunizing for example, mice with an immunogen. The mice are inoculated intra-peritoneally with an immunogenic amount of the above-described immunogen and then boosted with similar amounts of the immunogen. Spleens are collected from the immunized mice a few days after the final boost and a cell suspension is prepared from the spleens for use in the fusion.

In the practice of the subject invention any of the above-described antibodies may be labeled with a detectable marker. In one embodiment, the labeled antibody is a purified labeled antibody. As used in the subject invention, the term "antibody" includes, but is not limited to, both naturally occurring and non-naturally occurring antibodies. Specifically, the

term "antibody" includes polyclonal and monoclonal antibodies, and binding fragments thereof. Furthermore, the term "antibody" includes chimeric antibodies and wholly synthetic antibodies, and fragments thereof.

Furthermore, the term "antibody" includes chimeric antibodies and wholly synthetic antibodies, and fragments thereof. A "detectable moiety" which functions as detectable labels are well known to those of ordinary skill in the art and include, but are not limited to, a fluorescent label, a radioactive atom, a paramagnetic ion, biotin, a chemiluminescent label or a label which may be detected through a secondary enzymatic or binding step. The secondary enzymatic or binding step may comprise the use of digoxigenin, alkaline phosphatase, horseradish peroxidase,  $\beta$ -galactosidase, fluorescein or streptavidin/biotin. Methods of labeling antibodies are well known in the art.

Determining whether the antibody forms such a complex may be accomplished according to methods well known to those skilled in the art. In the preferred embodiment, the determining is accomplished according to flow cytometry methods.

The antibody may be bound to an insoluble matrix such as that used in affinity chromatography. As used in the subject invention, isolating the cells which form a complex with the immobilized monoclonal antibody may be achieved by standard methods well known to those skilled in the art. For example, isolating may comprise affinity chromatography using immobilized antibody.

Alternatively, the antibody may be a free antibody. In this case, isolating may comprise cell sorting using free, labeled primary or secondary antibodies. Such cell sorting methods are standard and are well known to those skilled in the art.

The labeled antibody may be a polyclonal or monoclonal antibody. In one embodiment, the labeled antibody is a purified labeled antibody. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies. Specifically, the term "antibody" includes polyclonal and monoclonal antibodies, and fragments thereof. Furthermore, the term "antibody" includes chimeric antibodies and wholly synthetic antibodies, and fragments thereof. The detectable marker may be, for example, radioactive or fluorescent. Methods of labeling antibodies are well known in the art.

This invention provides a method of inducing apoptosis in cells which comprises expressing polypeptide capable of binding a p75<sup>NTR</sup> receptor in the cells.

This invention provides a method of inducing apoptosis in a subject which comprises expressing a polypeptide capable of binding a p75<sup>NTR</sup> receptor in the subject. In a further embodiment of the method of inducing apoptosis in a subject where the subject is a rat, mouse or human.

As used herein, "subject" means any animal or artificially modified animal. Artificially modified animals include, but are not limited to, SCID mice with human immune systems. In the preferred embodiment, the subject is a human.

This invention provides a transgenic nonhuman mammal which comprises an isolated nucleic acid, encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor, which is a DNA molecule. In an embodiment of the above  
5 described transgenic nonhuman mammal, the DNA encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor is operatively linked to tissue specific regulatory elements.

10 This invention provides a method of determining physiological effects of expressing varying levels of a polypeptide capable of binding a p75<sup>NTR</sup> receptor in a transgenic nonhuman mammal which comprises producing a panel of transgenic nonhuman mammals, each nonhuman  
15 mammal expressing a different amount of a polypeptide capable of binding a p75<sup>NTR</sup> receptor.

This invention provides a method of producing a polypeptide capable of binding a p75<sup>NTR</sup> receptor into a suitable vector which comprises: (a) inserting a nucleic acid molecule encoding the polypeptide capable  
20 of binding a p75<sup>NTR</sup> receptor into a suitable vector; (b) introducing the resulting vector into a suitable host cell; (c) selecting the introduced host cell for the expression of the polypeptide capable of binding a p75<sup>NTR</sup> receptor; (d) culturing the selected cell to produce the polypeptide capable of binding a p75<sup>NTR</sup>  
25 receptor; and (e) recovering the polypeptide capable of binding a p75<sup>NTR</sup> receptor produced.

30 This invention provides a method of inducing apoptosis of cells in a subject comprising administering to the subject the purified polypeptide capable of binding a p75<sup>NTR</sup> receptor in an amount effective to induce  
35 apoptosis. In an embodiment of the above described

method of inducing apoptosis of cells in a subject comprising administering to the subject the purified polypeptide capable of binding a p75<sup>NTR</sup> receptor in an amount effective to induce apoptosis, the subject is  
5 a mammal. In another embodiment of the above-described method of inducing apoptosis of cells in a subject, the subject is a mouse, rat or human.

As used herein "apoptosis" means programmed cell death  
10 of the cell. The mechanisms and effects of programmed cell death differs from cell lysis. Some observable effects of apoptosis are: DNA fragmentation and disintegration into small membrane-bound fragments called apoptotic bodies.

As used herein, "subject" means any animal or  
15 artificially modified animal. Artificially modified animals include, but are not limited to, SCID mice with human immune systems. In the preferred  
20 embodiment, the subject is a human.

This invention provides a pharmaceutical composition comprising a purified polypeptide capable of binding  
25 a p75<sup>NTR</sup> receptor and a pharmaceutically acceptable carrier.

The invention also provides a pharmaceutical composition comprising a effective amount of the polypeptides capable of binding a p75<sup>NTR</sup> receptor  
30 described above and a pharmaceutically acceptable carrier. In the subject invention an "effective amount" is any amount of above-described polypeptides capable of binding a p75<sup>NTR</sup> receptor which, when administered to a subject suffering from a disease or  
35 abnormality against which the proteins are determined to be potentially therapeutic, are effective, causes

reduction, remission, or regression of the disease or abnormality. In the practice of this invention the "pharmaceutically acceptable carrier" is any physiological carrier known to those of ordinary skill  
5 in the art useful in formulating pharmaceutical compositions.

In one preferred embodiment the pharmaceutical carrier may be a liquid and the pharmaceutical composition  
10 would be in the form of a solution. In another equally preferred embodiment, the pharmaceutically acceptable carrier is a solid and the composition is in the form of a powder or tablet. In a further embodiment, the pharmaceutical carrier is a gel and  
15 the composition is in the form of a suppository or cream. In a further embodiment the compound may be formulated as a part of a pharmaceutically acceptable transdermal patch.

20 A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In  
25 powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in  
30 the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose,  
35 polyvinylpyrrolidone, low melting waxes and ion

exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by for example, intramuscular, intrathecal, epidural, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compounds may be prepared as a sterile solid



composition which may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium. Carriers are intended to include necessary and inert binders, suspending agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings.

The above described pharmaceutical composition comprising a polypeptide capable of binding a p75<sup>NTR</sup> receptor can be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents, for example, enough saline or glucose to make the solution isotonic, bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like.

The above described pharmaceutical composition comprising a polypeptide capable of binding a p75<sup>NTR</sup> receptor can also be administered orally either in liquid or solid composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular above described pharmaceutical composition comprising a polypeptide capable of binding a p75<sup>NTR</sup> receptor in use, the strength of the preparation, the mode of administration, and the advancement of the

disease condition or abnormality. Additional factors depending on the particular subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

5

As used herein, administering may be effected or performed using any of the various methods known to those skilled in the art. The administration may be intravenous, intraperitoneal, intrathecal, intralymphatic, intramuscular, intralesional, parenteral, epidural, subcutaneous; by infusion, liposome-mediated delivery, aerosol delivery; topical, oral, nasal, anal, ocular or otic delivery.

15 A method of identifying a compound capable of inhibiting binding between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor comprising: a) contacting the compound with the polypeptide capable of binding to p75<sup>NTR</sup> receptor under conditions permitting the binding of the polypeptide capable of binding to p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> receptor to form a complex; b) contacting the p75<sup>NTR</sup> receptor with the mixture from step a); and c) measuring the amount of the formed complexes or the unbound p75<sup>NTR</sup> receptor or the unbound polypeptide or any combination thereof. In an embodiment of the above described method of identifying a compound capable of inhibiting between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup> is a neurotrophin associated cell death executor. In an embodiment of the above described method of identifying a compound capable of inhibiting between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup> is a human HGR74 protein. In an embodiment of the above described method of

identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a musnade3a sequence as defined on Figure 1H. In  
5 an embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a hunade3a1 sequence as defined on Figure 1H. In  
10 an embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
a hunade3a2 sequence as defined on Figure 1H. In an  
15 embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
a ratnad3a sequence as defined on Figure 1H. In an  
20 embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a ratnad3b sequence as defined on Figure 1H. In an  
25 embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a musnade3b sequence as defined on Figure 1H. In  
30 an embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a humnadel sequence as defined on Figure 1H. In an  
35 embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding

5 p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a ratnadel sequence as defined on Figure 1H. In an  
embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a musnadel sequence as defined on Figure 1H. In  
an embodiment of the above described method of  
identifying a compound capable of inhibiting between  
10 p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a humnade2 sequence as defined on Figure 1H.

15 A method of identifying a compound capable of  
inhibiting binding between p75<sup>NTR</sup> receptor and a  
polypeptide capable of binding p75<sup>NTR</sup> receptor, where  
said binding forms a complex between p75<sup>NTR</sup> receptor  
and a polypeptide capable of binding p75<sup>NTR</sup> receptor,  
comprising: a) contacting the compound with the p75<sup>NTR</sup>  
20 receptor under conditions permitting the binding of  
the polypeptide capable of binding to p75<sup>NTR</sup> receptor  
and p75<sup>NTR</sup> receptor to form a complex; b) contacting  
the p75<sup>NTR</sup> receptor with the mixture from step a); and  
c) measuring the amount of the formed complexes or the  
25 unbound p75<sup>NTR</sup> receptor or the unbound polypeptide or  
any combination thereof.

In an embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
30 p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a neurotrophin associated cell death executor  
protein. In an embodiment of the above described  
method of identifying a compound capable of inhibiting  
between p75<sup>NTR</sup> receptor and a polypeptide capable of  
35 binding p75<sup>NTR</sup> where said polypeptide capable of  
binding p75<sup>NTR</sup> is a human HGR74 protein. In an  
embodiment of the above described method of

identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a musnade3a sequence as defined on Figure 1H. In  
5 an embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a hunade3a1 sequence as defined on Figure 1H. In  
10 an embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
a hunade3a2 sequence as defined on Figure 1H. In an  
15 embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
a ratnad3a sequence as defined on Figure 1H. In an  
20 embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a ratnad3b sequence as defined on Figure 1H. In an  
25 embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a musnade3b sequence as defined on Figure 1H. In  
30 an embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding  
p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup>  
is a humnade1 sequence as defined on Figure 1H. In an  
35 embodiment of the above described method of  
identifying a compound capable of inhibiting between  
p75<sup>NTR</sup> receptor and a polypeptide capable of binding

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p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup> is a ratnad1 sequence as defined on Figure 1H. In an embodiment of the above described method of identifying a compound capable of inhibiting between  
5 p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup> is a musnad1 sequence as defined on Figure 1H. In an embodiment of the above described method of identifying a compound capable of inhibiting between  
10 p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> where said polypeptide capable of binding p75<sup>NTR</sup> is a humnade2 sequence as defined on Figure 1H.

This invention provides a method for identifying an  
15 apoptosis inducing compound comprising: (a) contacting a subject with an appropriate amount of the compound; and (b) measuring the expression level of polypeptide capable of binding a p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene in the subject, an increase of the  
20 expression levels of a polypeptide capable of binding a p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound. In an embodiment of the above described method for identifying an apoptosis inducing compound comprising:  
25 a) contacting a subject with an appropriate amount of the compound; and (b) measuring the expression level of polypeptide capable of binding a p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene in the subject, an increase of the expression levels of a polypeptide capable of binding  
30 a p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound, wherein the subject is a mammal. In an embodiment of the above-described method of identifying an apoptosis inducing compound, wherein the mammal subject is a  
35 mouse, rat or human.

This invention provides a method for identifying an apoptosis inducing compound comprising: (a) contacting a cell with an appropriate amount of the compound; and (b) measuring the expression level of polypeptide capable of binding a p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene in the cell, an increase of the expression levels of polypeptide capable of binding a p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound.

10

An apoptosis inducing compound is defined as a compound which may be, but not limited to, antibodies, inorganic compounds, organic compounds, peptides, peptidomimetic compounds, polypeptides or proteins, fragments or derivatives which share some or all properties, e.g. fusion proteins, that induces apoptosis. The compounds may be naturally occurring and obtained by purification, or may be non-naturally occurring and obtained by synthesis.

20

This invention provides a method for screening cDNA libraries encoding a polypeptide capable of binding a p75<sup>NTR</sup> receptor sequence using a yeast two-hybrid system and using a p75<sup>NTR</sup> intracellular domain as a target. In an embodiment of the above described method for screening cDNA libraries for polypeptide capable of binding a p75<sup>NTR</sup> receptor sequence using a yeast two-hybrid system and using a p75<sup>NTR</sup> intracellular domain as a target, where the cDNA library is mammalian. In another embodiment of the above described method for screening cDNA libraries for a polypeptide capable of binding a p75<sup>NTR</sup> receptor using a yeast two-hybrid system and using a p75<sup>NTR</sup> intracellular domain as a target, where the cDNA library is mammalian and where the mammalian cDNA

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library is derived from rat, mouse or human cDNA libraries. In an embodiment of the above described method for screening cDNA libraries for a polypeptide capable of binding a p75<sup>NTR</sup> receptor, using a yeast  
5 two-hybrid system and using a p75<sup>NTR</sup> intracellular domain as a target, where the p75<sup>NTR</sup> intracellular domain target is mammalian. In an embodiment of the above described method for screening cDNA libraries for a polypeptide capable of binding a p75<sup>NTR</sup> receptor  
10 using a yeast two-hybrid system and using a p75<sup>NTR</sup> intracellular domain as a target, where the p75<sup>NTR</sup> intracellular domain target is a rat, mouse or human p75<sup>NTR</sup> intracellular domain target.

15 This invention provides a method to induce caspase-2 and caspase-3 activity to cleave poly (ADP-ribose) polymerase and fragment nuclear DNA in a cell by co-expression of a polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>.

20 Caspases are members of the protease family, the mammalian homologs of the *Caenorhabditis elegans* death gene ced-3, which are required for mammalian apoptosis. Increased levels of caspase-2 and caspase-  
25 3 have been linked to apoptosis. The caspases are cysteine aspartases that cleave their substrates at aspartate residues. To activate caspases, they need to be cleaved at aspartate residues and to form active heterodimers.

30 This invention provides a method to inhibit NF- $\kappa$ B activation in a cell with a polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>.

35 NF- $\kappa$ B is a primary transcription factor which is



activated by external stimuli, and translocated to the nucleus where it binds to DNA and regulates gene transcription. In rat Schwann cells, the binding of nerve growth factor to p75<sup>NTR</sup> neurotrophin receptor, induces the activation of NF- $\kappa$ B in the absence of tyrosine kinase receptor A, and led to cell survival. NF- $\kappa$ B regulates the gene expression of various proteins including cell surface molecules and cytokines.

This invention provides a method to detect a neurodegenerative disease in a subject by detecting expression levels of a polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>. In an embodiment of the above described method to detect a neurodegenerative disease in a subject by detecting expression levels of a polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>, wherein the subject is a mammal. In another embodiment of the above described method to detect a neurodegenerative disease in a subject by detecting expression levels of a polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> wherein the mammal subject is mouse, rat or human.

This invention provides a transgenic nonhuman mammal which comprises an isolated nucleic acid, encoding a human HGR74 protein, which is a DNA molecule. In an embodiment of the above described transgenic nonhuman mammal, the DNA encoding a human HGR74 protein is operatively linked to tissue specific regulatory elements.

This invention provides a method of determining physiological effects of expressing varying levels of a human HGR74 protein in a transgenic nonhuman mammal

which comprises producing a panel of transgenic nonhuman mammal, each nonhuman mammal expressing a different amount of human HGR74 protein.

5 This invention provides a method of producing the isolated human HGR74 protein into a suitable vector which comprises: (a) inserting a nucleic acid molecule encoding a human HGR74 protein into a  
10 suitable vector; (b) introducing the resulting vector into a suitable host cell; (c) selecting the introduced host cell for the expression of the human HGR74 protein; (d) culturing the selected cell to produce the human HGR74 protein; and (e) recovering the human HGR74 protein produced.

15 This invention provides a method of inducing apoptosis of cells in a subject comprising administering to the subject the purified human HGR74 protein in an amount effective to induce apoptosis. In an embodiment of  
20 the above described method of inducing apoptosis of cells in a subject comprising administering to the subject the purified human HGR74 in an amount effective to induce apoptosis, the subject is a mammal. In another embodiment of the above-described  
25 method of inducing apoptosis of cells in a subject, the subject is a mouse, rat or human.

This invention provides a pharmaceutical composition comprising a purified human HGR74 protein and a  
30 pharmaceutically acceptable carrier.

This invention provides a method for identifying an apoptosis inducing compound comprising: (a)  
35 contacting a subject with an appropriate amount of the compound; and (b) measuring the expression level of

human HGR74 protein gene and p75<sup>NTR</sup> gene in the subject, an increase of the expression levels of human HGR74 protein gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound. In an embodiment of the above described method for identifying an apoptosis inducing compound comprising: a) contacting a subject with an appropriate amount of the compound; and (b) measuring the expression level of human HGR74 protein gene and p75<sup>NTR</sup> gene in the subject, an increase of the expression levels of human HGR74 protein gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound, wherein the subject is a mammal. In an embodiment of the above-described method of identifying an apoptosis inducing compound, wherein the mammal subject is a mouse, rat or human.

This invention provides a method for identifying an apoptosis inducing compound comprising: (a) contacting a cell with an appropriate amount of the compound; and (b) measuring the expression level of human HGR74 gene and p75<sup>NTR</sup> gene in the cell, an increase of the expression levels of human HGR74 protein gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound.

This invention provides a method for screening cDNA libraries human HGR74 sequence using a yeast two-hybrid system using a p75<sup>NTR</sup> intracellular domain as a target. In an embodiment of the above described method for screening cDNA libraries human HGR74 sequence using a yeast two-hybrid system using a p75<sup>NTR</sup> intracellular domain as a target, where the cDNA library is mammalian. In an embodiment of the above described method for screening cDNA libraries human

HGR74 sequence using a yeast two-hybrid system using a p75<sup>NTR</sup> intracellular domain as a target, where the cDNA library is mammalian and where the mammalian cDNA library is derived from rat, mouse or human cDNA libraries. In another embodiment of the above described method for screening cDNA libraries human HGR74 sequence using a yeast two-hybrid system using a p75<sup>NTR</sup> intracellular domain as a target, where the p75<sup>NTR</sup> intracellular domain target is mammalian. In an embodiment of the above described method for screening cDNA libraries human HGR74 sequence using a yeast two-hybrid system using a p75<sup>NTR</sup> intracellular domain as a target, where the p75<sup>NTR</sup> intracellular domain target is a rat, mouse or human p75<sup>NTR</sup> intracellular domain target.

This invention provides a method to induce caspase-2 and caspase-3 activity to cleave poly (ADP-ribose) polymerase and fragment nuclear DNA in a cell by co-expression of human HGR74 protein and p75<sup>NTR</sup>.

This invention provides a method to inhibit NF- $\kappa$ B activation in a cell with human HGR74 protein and p75<sup>NTR</sup>.

This invention provides a method to detect a neurodegenerative disease in a subject by detecting expression levels of polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>. In an embodiment of the above described method to detect a neurodegenerative disease in a subject by detecting expression levels of polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>, wherein the subject is a mammal. In another embodiment of the above described method to detect a neurodegenerative disease in a subject by detecting

expression levels of polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>, wherein the subject is a mammal wherein the mammal is human.

5 This invention provides a method of identifying a compound, which is an apoptosis inhibitor, said compound is capable of inhibiting specific binding between a polypeptide capable of binding a p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> receptor, so as to prevent  
10 apoptosis which comprises: (a) contacting the polypeptide capable of binding a p75<sup>NTR</sup> receptor with a plurality of compounds under conditions permitting binding between a known compound previously shown to be able to displace the polypeptide capable of binding  
15 a p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor and the bound p75<sup>NTR</sup> receptor to form a complex; and (b) detecting the displaced polypeptide capable of binding a p75<sup>NTR</sup> receptor or the complex formed in step (a), wherein the displacement indicates that the compound is  
20 capable of inhibiting specific binding between the polypeptide capable of binding a p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor. In another embodiment of the above described method, wherein the inhibition of specific binding between the polypeptide capable of binding a  
25 p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor affects the transcription activity of a reporter gene. In a further embodiment of the above described method, wherein step (b) the displaced polypeptide capable of binding a p75<sup>NTR</sup> receptor or the complex is detected by  
30 comparing the transcription activity of a reporter gene before and after the contacting with the compound in step (a), where a change of the activity indicates that the specific binding between the polypeptide capable of binding a p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup>  
35 receptor is inhibited and the polypeptide capable of

binding a p75<sup>NTR</sup> receptor is displaced. In an embodiment of the above described method, wherein the p75<sup>NTR</sup> receptor is bound to a solid support. In a further embodiment of the above described method, wherein the compound is bound to a solid support. In an embodiment of the above described method, wherein the compound comprises an antibody, an inorganic compound, an organic compound, a peptide, a peptidomimetic compound, a polypeptide or a protein. In an embodiment of the above described method, wherein the contacting of step (a) is in vitro. In a further embodiment of the above method, wherein the contacting of step (a) is in vivo. In an embodiment of the above method, wherein the contacting of step (a) is in a yeast cell. In an embodiment of the above method, wherein the contacting or step (a) is in a mammalian cell. In an embodiment of the above method, wherein the polypeptide capable of binding a p75<sup>NTR</sup> receptor is a cell surface receptor. In an embodiment of the above method, wherein the cell-surface receptor is the p75 receptor.

As used herein, the "transcription activity of a reporter gene" means that the expression level of the reporter gene will be altered from the level observed when the signal-transducing protein and the cytoplasmic protein are bound. One can also identify the compound by detecting other biological functions dependent on the binding between the signal-transducing protein and the cytoplasmic protein. Examples of reporter genes are numerous and well-known in the art, including, but not limited to, histidine resistant genes, ampicillin resistant genes,  $\beta$ -galactosidase gene.

Further the cytoplasmic protein may be bound to a solid support. Also the compound may be bound to a solid support and comprises an antibody, an inorganic compound, an organic compound, a peptide, a peptidomimetic compound, a polypeptide or a protein.

An example of the method is provided infra. One can identify a compound capable of inhibiting specific binding between the signal-transducing protein and the cytoplasmic protein using direct methods of detection such as immuno-precipitation of the cytoplasmic protein and the compound bound to a detectable marker. Further, one could use indirect methods of detection that would detect the increase or decrease in levels of gene expression. As discussed infra, one could construct synthetic peptides fused to a LexA DNA binding domain. These constructs would be transformed into the L40-strain with an appropriate cell line having an appropriate reporter gene. One could then detect whether inhibition had occurred by detecting the levels of expression of the reporter gene. In order to detect the expression levels of the reporter gene, one skilled in the art could employ a variety of well-known methods, e.g. two-hybrid systems in yeast, mammals or other cells.

Further, the contacting of step (a) may be in vitro, in vivo, and specifically in an appropriate cell, e.g. yeast cell or mammalian cell. Examples of mammalian cells include, but not limited to, the mouse fibroblast cell NIH 3T3, CHO cells, HeLa cells, Ltk<sup>-</sup> cells, Cos cells, etc.

Other suitable cells include, but are not limited to, prokaryotic or eukaryotic cells, e.g. bacterial cells

(including gram positive cells), fungal cells, insect cells, and other animals cells.

5 This invention provides a method of identifying a compound, which is an apoptosis inhibitor, said compound is capable of inhibiting specific binding between human HGR74 protein and p75<sup>NTR</sup> receptor, so as to prevent apoptosis which comprises: (a) contacting the human HGR74 protein with a plurality of compounds  
10 under conditions permitting binding between a known compound previously shown to be able to displace the human HGR74 protein and the p75<sup>NTR</sup> receptor and the bound p75<sup>NTR</sup> receptor to form a complex; and (b) detecting the displaced human HGR74 protein or the  
15 complex formed in step (a), wherein the displacement indicates that the compound is capable of inhibiting specific binding between the human HGR74 protein and the p75<sup>NTR</sup> receptor. In an embodiment of the above described method, wherein the inhibition of specific  
20 binding between the human HGR74 protein and the p75<sup>NTR</sup> receptor affects the transcription activity of a reporter gene. In a further embodiment of the above described method, wherein step (b) the displaced human HGR74 protein or the complex is detected by comparing  
25 the transcription activity of a reporter gene before and after the contacting with the compound in step (a), where a change of the activity indicates that the specific binding between the human HGR74 protein and the p75<sup>NTR</sup> receptor is inhibited and the human HGR74  
30 protein is displaced. In an embodiment of the above described method, wherein the p75<sup>NTR</sup> receptor is bound to a solid support. In a further embodiment of the above described method, wherein the compound is bound to a solid support. In an embodiment of the above  
35 described method, wherein the compound comprises an



antibody, an inorganic compound, an organic compound,  
a peptide, a peptidomimetic compound, a polypeptide or  
a protein. In an embodiment of the above described  
method, wherein the contacting of step (a) is in  
5 vitro. In a further embodiment of the above method,  
wherein the contacting of step (a) is in vivo. In an  
embodiment of the above method, wherein the contacting  
of step (a) is in a yeast cell. In an embodiment of  
the above method, wherein the contacting or step (a)  
10 is in a mammalian cell. In an embodiment of the above  
method, wherein the human HGR74 protein is a cell  
surface receptor. In an embodiment of the above  
method, wherein the cell-surface receptor is the p75  
receptor.

15 As used herein, the "transcription activity of a  
reporter gene" means that the expression level of the  
reporter gene will be altered from the level observed  
when the signal-transducing protein and the  
20 cytoplasmic protein are bound. One can also identify  
the compound by detecting other biological functions  
dependent on the binding between the signal-  
transducing protein and the cytoplasmic protein.  
Examples of reporter genes are numerous and well-known  
25 in the art, including, but not limited to, histidine  
resistant genes, ampicillin resistant genes,  $\beta$ -  
galactosidase gene.

30 Further the cytoplasmic protein may be bound to a  
solid support. Also the compound may be bound to a  
solid support and comprises an antibody, an inorganic  
compound, an organic compound, a peptide, a  
peptidomimetic compound, a polypeptide or a protein.

35 An example of the method is provided infra. One can

identify a compound capable of inhibiting specific binding between the signal-transducing protein and the cytoplasmic protein using direct methods of detection such as immuno-precipitation of the cytoplasmic protein and the compound bound to a detectable marker. Further, one could use indirect methods of detection that would detect the increase or decrease in levels of gene expression. As discussed infra, one could construct synthetic peptides fused to a LexA DNA binding domain. These constructs would be transformed into the L40-strain with an appropriate cell line having an appropriate reporter gene. One could then detect whether inhibition had occurred by detecting the levels of expression of the reporter gene. In order to detect the expression levels of the reporter gene, one skilled in the art could employ a variety of well-known methods, e.g. two-hybrid systems in yeast, mammals or other cells.

Further, the contacting of step (a) may be in vitro, in vivo, and specifically in an appropriate cell, e.g. yeast cell or mammalian cell. Examples of mammalian cells include, but not limited to, the mouse fibroblast cell NIH 3T3, CHO cells, HeLa cells, Ltk<sup>-</sup> cells, Cos cells, etc.

Other suitable cells include, but are not limited to, prokaryotic or eukaryotic cells, e.g. bacterial cells (including gram positive cells), fungal cells, insect cells, and other animals cells.

In order to facilitate an understanding of the material which follows, certain frequently occurring methods and/or terms are best described in Sambrook, et al., 1989.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely  
5 illustrative of the invention as described more fully in the claims which follow thereafter.

5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 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## Experimental Details

### **Results and Discussions**

5 The p75<sup>NTR</sup> is the first-isolated neurotrophin receptor  
and the member of TNFR (tumor necrosis factor  
receptor) family (7, 8). However, its functional role  
and signaling pathway has remained largely unclear  
(9). The existence of p75<sup>NTR</sup>ICD binding proteins have  
10 been implicated since p75<sup>NTR</sup>ICD does not have a typical  
biochemical motif except a C-terminal region well  
conserved to a type 2 death domain (10). Recently, it  
has been reported that TRAF6 is involved in p75<sup>NTR</sup>-  
mediated signal transduction(11). To further identify  
the p75<sup>NTR</sup>ICD binding proteins, we screened the mouse  
15 cDNA libraries by yeast two-hybrid system using a rat  
p75<sup>NTR</sup>ICD as a target and one of positive clones was  
identified as a p75<sup>NTR</sup>-associated cell death executor,  
NADE.

20 NADE consists of 124 amino acids and its molecular  
weight is calculated to 14,532 dalton. NADE is a  
hydrophilic and acidic protein, and the estimated pI  
value is 5.97. A BLAST search revealed that NADE has  
significant homology to a known human protein HGR74(4)  
25 (Fig. 1a), and does not have a significant motif  
except the leucine rich nuclear export signal (NES)  
(5) (Fig. 1b) and ubiquitination sequences (6) (Fig.  
1c) HGR74 was previously reported as an abundant mRNA  
expressed in human ovarian granulosa cells, however,  
30 its functional role is still unknown. The homology of  
these two proteins except the asparagine rich stretch  
(a. a. 36-48) of NADE is 92.8%, therefore we conclude  
that HGR74 is a human homolog of mouse NADE.

35 Northern blot analysis is revealed that NADE mRNA (1.3  
kbp) is found highest in several tissues including  
brain, heart, and lung (Fig. 1d). We could also

detect a low level of mRNA expression in stomach, small intestine, and muscle by a long exposure (data not shown). But there was no expression in liver. The additional large band (3.0 kbp) was also observed

5 in testis, suggesting the existence of the alternative splicing form. The endogenous NADE protein was also confirmed in human neuroblastoma cell line, SK-N-MC by immunoprecipitation using the anti-NADE antibody (Fig. 1e). Interestingly, in SK-N-MC, PC12 and PCNA cells,

10 NADE protein can be detected only in the presence of the ubiquitin inhibitor such as ALLN, suggesting that NADE is modified by ubiquitin conjugating system leading to subsequent degradation by the proteasome. The molecular size of NADE is estimated to 22 kDa by

15 the SDS-PAGE, and this size seems to be slightly larger than the molecular weight predicted from nucleotide sequence. But the gap of molecular size might be caused by its low pI value or post-translational modification in a potential prenylation site (Fig. 1a). The overexpressed NADE protein in

20 293T cells showed the two bands, 22 kDa and 44 kDa in SDS-PAGE under the reduced condition at 100 mM dithiothreitol (Fig. 1f). To clarify this question, two NADE mutants were constructed and expressed in

25 293T cells. Since NADE has two cysteine residues at sequence positions 102 and 121, we replaced the each cysteine with the serine residue. Western blot analysis revealed that the molecular weight of muNADE (Cys121Ser) is identical to a wild type, on the other

30 hand, muNADE (Cys102Ser) showed the only smaller band of 22 kDa (Fig. 1g). These results strongly suggested that NADE can heterodimerize by the disulfide bond at the Cys102, and resulted in the 44 kDa band.

35 *In vitro*-translated mouse NADE protein and *E. coli*-expressed GST- p75<sup>NTR</sup>ICD fusion protein were used for *in vitro* GST pull down assay. In this assay, the NADE

protein showed the strong binding activity to GST-P  
p75<sup>NTR</sup>ICD (Fig. 2a). To investigate the *in vivo*  
binding activity, the Myc-tagged NADE and p75<sup>NTR</sup> were  
co-expressed in 293T cells and subjected to the co-  
immunoprecipitation experiment. The results clearly  
showed that NADE could bind to a full length of p75<sup>NTR</sup>  
*in vivo* very strongly (Fig. 2b) and the recruitment of  
NADE protein to p75<sup>NTR</sup>ICD was detected in a dose  
dependent of NGF (Fig. 2c), suggesting that NADE  
protein is a putative signal transducing protein  
interacting with p75<sup>NTR</sup>ICD. Furthermore, our mapping  
studies revealed that NADE protein interacts with the  
cell death domain (amino acid residues 338-393) which  
is identical among mouse, rat and human (data not  
shown). Since TRAF6 binds a conserved juxtamembrane  
region (11), it is unlikely that NADE protein inhibits  
TRAF6 binding to p75<sup>NTR</sup>. It has been speculated that  
the polymerization of p75<sup>NTR</sup> is important for its  
signal transduction similar to the another members of  
TNFR family. For example, TNFRI (12), CD40 (13), and  
Fas (14) are formed the trimer through the binding of  
each trimer ligands to extracellular domain. However,  
there was no previous report for p75<sup>NTR</sup> in same manner  
(15). It may be possible that the dimer formation of  
p75<sup>NTR</sup> occurs through the binding of NADE dimer to its  
intracellular domain.

To investigate the functional role of NADE protein,  
NADE and p75<sup>NTR</sup> were co-transfected in 293T cells. The  
results showed that the co-transfected 293T cells were  
detached from the dish and aggregated 48 hours later  
(Fig. 3a). However, 293T cells transfected with the  
control plasmid DNAs showed no significant differences  
(Fig. 3a), implicating that this morphological change  
is caused by apoptosis. We further examined the TUNEL  
assay (TdT-mediated dUTP-biotin nick end labeling  
assay) (16) as well as the DNA fragmentation test on

these cells. On the TUNEL assay, the significant increase of dying cell was detected only in co-transfected cells (Fig. 3b) and the value of the positive cell percentage (38%) was consistent with the transfection efficiency by the calcium-phosphate method. Furthermore, the DNA fragmentation was detected in only the co-transfected 293T cells (Fig. 3c). From these results, we conclude that the co-expression of NADE and p75<sup>NTR</sup> induced apoptosis in 293T cells.

Although NADE protein is recruited to the cytoplasmic region of p75<sup>NTR</sup> in a ligand-dependent manner, NGF-dependent cell death was not clearly detected in the co-transfected 293T cells in the presence of NGF (100 ng/ml) (data not shown), suggesting that NADE protein may function in the p75<sup>NTR</sup>-mediated cell death machinery to transduce the downstream signal to apoptosis independent on NGF.

To further investigate the physiological function of NADE protein, we checked the transcription factor kappa B (NF-kB), Caspase-2, and Caspase-3 activities in 293T cells co-transfected with NADE and p75<sup>NTR</sup>. NF-kB is activated by external stimuli, and translocated to the nucleus where it binds to DNA and regulates gene transcription (17). In rat Schwann cells, the binding of NGF to p75<sup>NTR</sup> induces the activation of NF-kB with independent manner of TrkA (18) leading to the cell survival and TRAF6 may be a component of NGF-mediated NF-kB activation (11). In contrast, expression of NADE protein significantly suppressed the NF-kB activity in a dose dependent manner, but this effect was not markedly co-operative with p75<sup>NTR</sup> expression (Fig. 3d) as well as NGF-dependent manner (data not shown), implicating that p75<sup>NTR</sup>/NADE-induced apoptosis may not be due to only the suppression of

NF-kB activity but also the regulation of unknown signal molecules since NF-kB suppression by NADE protein alone could not induce apoptosis. It has been reported that suppression of NF-kB activity increases cell death in PC12 cells expressing p75<sup>NTR</sup> (19, 20). NADE protein may play a key role in the downregulation of NF-kB activity and ultimately lead to apoptosis in neuronal cells expressing p75<sup>NTR</sup>.

In many cases of apoptosis, the elevation of Caspase-3 activity was observed (21, 22, 23, 24). This protease normally exists in cytosol of cells as 32 kDa precursor that is proteolytically activated into a 20 kDa and a 10 kDa heterodimer when cells are signaled to undergo apoptosis in response to serum withdrawal, activation of Fas, treatment with ionization, and a variety of pharmacological agents (25). Western blot analysis revealed that Caspase-2 and Caspase-3 were significantly processed only in 293T cells co-transfected with NADE and p75<sup>NTR</sup> (Fig. 3e). Moreover, PARP (poly (ADP-ribose) polymerase) which is a substrate for both Caspase-2 and Caspase-3 were partially cleaved, indicating that these Caspases are involved in apoptosis mediated by p75<sup>NTR</sup>/NADE signal transduction

To investigate whether NES sequences (5) contained in NADE (Fig. 4a) have the capability to export a protein from the nucleus to the cytosol, we performed the transient expression in 293T cells using a series of NADE mutants. The results indicated that NADE proteins with NES sequences localize in the cytoplasmic region (Fig. 4, lower panels of b, upper panels of c and d), but NADE proteins with NES mutations express in the nucleus (Fig. 4, lower panel of c and d). These data support the hypothesis that NADE protein can be exported from the nucleus to the



cytosol and may be post-translationally modified as a prenylated protein to promote and regulate p75<sup>NTR</sup>/NADE physiological interaction.

5 The signal cascade mediated by p75<sup>NTR</sup> has been enigmatic for a long time. But the recent growing evidences indicate that, not like other members of TNFR family, p75<sup>NTR</sup> can bifunctionally mediate signals to induce and inhibit apoptosis (26, 27). Our results  
10 strongly supported that NADE is a putative signal transducer for p75<sup>NTR</sup>-mediated apoptosis. Although NADE can mediate apoptosis cooperative with p75<sup>NTR</sup>, it is possible that NADE may be a signal adaptor molecule to interact with another effector molecules in p75<sup>NTR</sup>-  
15 mediated signal transduction. More importantly, since NADE has nuclear export signal (NES) as well as ubiquitination sequence, NADE may be tightly controlled by the ubiquitin/proteasome to shuttle another molecule from the nucleus to the cytoplasm,  
20 implicating that NADE is a very important protein for turnover of regulator gene such as the cell cycle-related proteins. Further investigation under physiological condition will give us more insight to better understand the mechanisms by which NADE can  
25 induce apoptosis together with p75<sup>NTR</sup> expression.

## Methods

### Isolation of p75<sup>NTR</sup>-associated cell death executor (NADE) by yeast two-hybrid system.

30 In order to isolate cDNA encoding p75<sup>NTR</sup>-associated proteins, we used yeast two-hybrid system, originally developed by Fields and Song (28). We used the cytosolic domain of rat p75<sup>NTR</sup> cDNA corresponding to  
35 amino acids 338-396 (representing the cytosolic domain of the protein from the transmembrane domain to the C-terminus of the protein) as a target. This portion of

p75<sup>NTR</sup> cDNA was PCR-engineered into the yeast expression plasmid pBTM116 in-frame with sequences encoding the LexA DNA-binding domain (29). This plasmid was then introduced into L40 cells [a, his3, trp1, leu2, ade2, lys2: (lexAop)<sup>4</sup>-HIS3, URA3: (lexAop)<sup>8</sup>-lacZ] which contain histidine synthetase (HIS3) and b-galactosidase (lacZ) reporter genes under the control of lexA operators (29). After confirming the expression of LexA-p75<sup>NTR</sup> (338-396) protein by immunoblotting using an antiserum specific for LexA, a mouse embryo pVP16 cDNA libraries were then introduced into these LexA/p75<sup>NTR</sup>-expressing cells by a high efficiency LiOAc transformation method (30, 31, 32). From a screen of 5 x 10<sup>7</sup> transformants, an initial set of 672 His<sup>+</sup> colonies were identified. These 672 clones were then tested by a  $\beta$ -galactosidase colorimetric assay (33), utilizing the lacZ reporter gene under the control of 8 lexA operators, thus narrowing down the pool of candidate clones to 181. These 181 candidates were then "cured" of their LexA/p75<sup>NTR</sup>-encoding plasmids by growth in tryptophan containing media, and mated with a panel of Mata-type yeast strain NA87-11A [a, leu2, his3, trp1, pho3, pho5] into which we had introduced various control plasmids that produce LexA fusion proteins, including LexA/p75<sup>NTR</sup>, LexA/Ras, Lex/CD40, LexA/Fas, and LexA/lamin. Among the 181 candidate clones, 1 clone specifically reacted with the LexA/p75<sup>NTR</sup> protein was chosen for further analysis. This mouse cDNA clone No. 59 has insert sizes of 450 bp. Because of its ability to induce cell death with expression of p75<sup>NTR</sup>, we have named this protein, NADE (p75<sup>NTR</sup>-associated cell death executor).

#### 35 DNA construction.

A full length mouse NADE cDNA was constructed on pBluescript II vector by the ligation of the partial

NADE cDNA (7-524) and 5'-RACE product. PCR cloning techniques were used to replace the stop codon and add the 5' *XhoI* site and 3' *BamHI* site of a full length NADE cDNA. pcDNA3.1(-)Myc-HisA/NADE was constructed by  
5 insertion of a full length NADE cDNA to *XhoI*-*BamHI* site of pcDNA3.1(-)Myc-HisA (Invitrogen). Human NADE cDNA was amplified using a Jurkat T cell cDNA library and cloned to pcDNA3.1(-)Myc-HisA pcDNA3/rat p75<sup>NTR</sup> was constructed by insertion of a full length rat p75<sup>NTR</sup>  
10 cDNA to *EcoRI* site of pcDNA3 (Invitrogen). pGEX4T-1/rat p75<sup>NTR</sup>ICD was constructed by insertion of amplified rat p75<sup>NTR</sup>ICD(a. a. 338-396) to pGEX4T-1 (Pharmacia). Mutant NADE expression plasmids, pcDNA3.1(-)Myc-HisA/muNADE (Cys102Ser) and pcDNA3.1(-)  
15 )Myc-HisA/muNADE (Cys121Ser), were constructed by PCR-based site-direct mutagenesis methods (29). pELAM-Lu for luciferase reporter assay was constructed by insertion of NF- $\kappa$ B binding site of E-selectin promoter region (-730 - 52) to pGL3-Basic *SacI*-*BglIII* site.  
20 Expression plasmids of GFP-fused NADE proteins were made following: The cDNA of GFP was cloned into *NheI*-*XhoI*-cut pcDNA3.1-mouse NADE as a PCR product amplified with the primers 5'-CTAGCTAGCATCATGGTGAGCAAGGGCGAG-3" and 5'-CCGCTCGAGTCTTGACAGCTCGTCCAT-3" using pEGFP-N2  
25 (Clontech) as a template. The deletion mutants delta 101-124-GFP and delta 91-124-GFP were constructed by inserting an *XhoI*-*BamHI*-cut PCR fragment generated with Expand high fidelity Taq polimerase (Boehringer Mannheim) into *XhoI*-*BamHI*-cut pcDNA3.1-GFP using the  
30 primers  
5'-ATCCTCGAGCGATCATGGCCAATGTCCAC-3" (sense),  
5'-ATCGGATCCTCTCAGCTGTAGCTCCCT-3" (antisense) and  
5'-ATCGGATCCGATCTCTCATCTCCTC-3" (antisense).  
35  
The mutagenic primers  
(5'-AAAGCTTAGGGAGGCACAGCTGAGAAA-3",

5"-TTTCTCAGCTGTGCCTCCCTAAGCTTT-3",  
5"-ATCCGGAGAAAGGCTAGGGAGGCACA-3",  
and 5"-TGTGCCTCCCTAGCCTTTCTCCGGAT-3")

5 were used to obtain L97A-GFP and L94, 97A-GFP in which  
Leu94 and Leu97 are replaced with Ala. In all  
constructs, mutations were verified by sequencing.

**Northern blot analysis.** 400 ng of NADE cDNA fragments  
(nt. 5-510) were labeled by 50  $\mu$ Ci of [ $\alpha$ -<sup>32</sup>P]dCTP and  
10 used as a probe. Each 10  $\mu$ g of total mRNA extracted  
from mouse various tissues were transferred on  
membranes and they were hybridized with a NADE probe  
for 2 hours at 68 °C using a express hybrid buffer  
(Clontech) and washed with 2 x SSC, 0.05 % SDS for 5  
15 times, and 0.1 x SSC, 0.1 % SDS for 1 time.

**Antibodies.** The polyclonal anti-NADE antibody was  
prepared by immunization of GST-mouse NADE fusion  
protein into the rabbit. The NADE specific antibody  
20 was affinity purified by antigen coupled Sepharose 4B.  
The polyclonal anti-rat p75<sup>NTR</sup> was kindly gifted from  
Dr. M. V. Chao. The monoclonal anti-Myc antibody  
(9E10) was purchased from BIOMOL. The polyclonal  
anti-Caspase-3 antibody (H-277) was purchased from  
25 Santa Cruz Biotechnology. The polyclonal Caspase-2  
antibody was kindly gifted from Dr. Lloyd A. Greene.  
HRP conjugated anti-rabbit IgG was purchased from Bio-  
Rad.

30 **Immunoprecipitation and immunoblotting.** In Fig. 1e,  
150  $\mu$ g/ml of ALLN (N-Acetyl-Leu-Leu-Norleucinal)  
treated SK-N-MC cells ( $1 \times 10^7$ ) were lysed in 0.5 ml of  
RIPA buffer. The supernatant of centrifuge (100,000  
x g) was mixed with 1  $\mu$ g of polyclonal anti-NADE  
35 antibody coupled Sepharose 4B, and incubated for 4  
hours at 4 °C. After washing, the gels were boiled by  
30  $\mu$ l of SDS-PAGE sampling buffer and subjected to

12.5 % of SDS-PAGE. Immunoblotting was performed by polyclonal anti-NADE antibody (2  $\mu$ g/ml). In Fig. 1f, 10  $\mu$ g of cell lysate extracted from each transfected 293T cells were used for the detection of NADE by immunoblotting.

**Transfection and protein expression in 293T cell.** In Fig. 1f, 293T cells ( $2 \times 10^6$ ) were transfected by 10  $\mu$ g of pcDNA3.1(-)Myc-HisA/NADE, pcDNA3.1(-)Myc-HisA/muNADE (Cys102Ser), or pcDNA3.1(-)Myc-HisA/muNADE(Cys121Ser) by calcium-phosphate method. In Fig. 2 b, 3 a, b, c, e, 293T cells ( $2 \times 10^6$ ) were transfected by 20  $\mu$ g of pcDNA3.1(-) Myc-HisA, 10  $\mu$ g of pcDNA3/rat p75<sup>NTR</sup> and 10  $\mu$ g of pcDNA3.1(-) Myc-HisA, 10  $\mu$ g of pcDNA3.1(-)Myc-HisA NADE and 10  $\mu$ g of pcDNA3.1(-) Myc-HisA, or 10  $\mu$ g of pcDNA3.1(-)Myc-HisA/NADE and 10  $\mu$ g of pcDNA3 / rat p75<sup>NTR</sup>. In Fig. 2 c, 293T cells ( $2 \times 10^6$ ) were transfected by 10  $\mu$ g of pcDNA3.1(-)Myc-HisA/NADE and 10  $\mu$ g of pcDNA3/rat p75<sup>NTR</sup> in serum minus DMEM medium.

**In vitro binding assay.** 5  $\mu$ l of L-[<sup>35</sup>S] methionine labeled, and *in vitro*- translated NADE protein was mixed with 5  $\mu$ l of GST-rat p75<sup>NTR</sup>ICD fusion protein or GST-coupled GSH-Sepharose 4B (Pharmacia) in 100  $\mu$ l of NETN buffer (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.2 % NP-40) for 18 hours at 4 °C. After washing, gels were boiled by 30  $\mu$ l of SDS-PAGE sampling buffer and subjected to 13.5 % SDS-PAGE. The fluorography was performed for 16 hours at -70 °C.

**In vivo binding assay.** In Fig. 2b, transfected 293T cells were lysed in 1 ml of NETN buffer and centrifuged (100,000  $\mu$ g). The supernatants were immunoprecipitated by 2  $\mu$ g of anti-Myc antibody coupled Protein G Sepharose 4B (Pharmacia) for 2 hours at 4 °C. Following the 5 times washing, gels were

subjected to 7.5 % SDS-PAGE, and Western blot analysis by rabbit polyclonal anti-p75<sup>NTR</sup> antibody.

**Interaction of NADE with p75<sup>NTR</sup> dependent on NGF**  
5 **ligation.** After co-transfection, cells were incubated in DMEM medium containing various NGF. After 12 hours later, the interaction activity between NADE and p75<sup>NTR</sup> were checked by *in vivo* binding assay.

10 **TUNEL assay.** MEBSTAIN Apoptosis kit direct (MIC) was used for TUNEL assay and the assay was done according to the company instruction. The stained cells were analyzed by FACSCalibur flow cytometer (Becton Dickinson).

15 **DNA fragmentation assay.** Transfected 293T cells were lysed in 350  $\mu$ l of 10 mM EDTA and 0.5 % SDS for 10 minutes at room temperature. After adding 100  $\mu$ l of 5 M NaCl, the aliquot was incubated for 18 hours at 4  
20 °C and centrifuged (12,000 x g). The supernatants were treated by 1 mg/ml of RNase A and 50 ng/ml of Proteinase K for 2 hours at 42 °C. After the phenol-chloroform extraction, the DNAs were precipitated by 70 % ethanol, and dissolved in 30  $\mu$ l of H<sub>2</sub>O. 5  $\mu$ l of  
25 samples were subjected to the 1.5 % agarose gel electrophoresis.

**Measurement of NF- $\kappa$ B activity.** Dual-Luciferase Reporter Assay System (Promega) was used for  
30 measurement of NF- $\kappa$ B activity. 293T cells ( $4 \times 10^5$ ) were transfected with 1.5  $\mu$ g of pELAM-luc reporter plasmid, 0.1  $\mu$ g of pRL-TK, 0.7  $\mu$ g of pcDNA3 rat p75<sup>NTR</sup>, 0.3  $\mu$ g or 2.8  $\mu$ g of pcDNA3.1(-) Myc-HisA/NADE and enough pcDNA3.1(-) Myc-His a control plasmid to give  
35 5.1  $\mu$ g of total DNA. Luciferase activities were determined 24 hours after transfection and normalized on the basis of pRL-TK expression levels. The

luciferase activities were measured by Turner Designs Luminometer Model TD20/20 (Promega).

**Confocal laser microscopy**

- 5 Transient transfections were carried out using the calcium phosphate precipitation method. 293T cells ( $3 \times 10^5$ ) on a cover glass were transiently transfected with 3.0 ug of DNA. After 12-24 hours, cells were fixed with 4 % paraformaldehyde and stained with TO-  
10 PRO-3 Iodide (Molecular Probes, Inc.) to visualize the nucleus. The subcellular distribution of GFP fusion proteins was examined using confocal laser microscopy (Carl Zeiss LSM510).

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**What is claimed is:**

1. An isolated nucleic acid molecule encoding a polypeptide capable of binding with a p75<sup>NTR</sup> receptor.  
5
2. The isolated DNA molecule of claim 1.
3. The isolated cDNA molecule of claim 2.  
10
4. The isolated RNA molecule of claim 1.
5. The isolated nucleic acid molecule of claims 1-4 encoding a neurotrophin associated cell death executor protein.  
15
6. The isolated nucleic acid molecule of claims 1-4 which comprises a sequence of AATTG TCTAC GCATC CTTAT GGGGG AGCTG TCTAA C.  
20
7. The isolated nucleic acid molecule of claim 5 which comprises a sequence of AATTG TCTAC GCATC CTTAT GGGGG AGCTG TCTAA C.
8. The isolated nucleic acid of claim 1 operatively linked to a promoter of RNA transcription.  
25
9. A vector which comprises the isolated nucleic acid of claim 1, operatively linked to a promoter of RNA transcription.  
30
10. The vector of claim 9, wherein the vector is plasmid.
11. The isolated nucleic acid molecule of claim 3, wherein the nucleic acid molecule encodes human or mouse polypeptide capable of binding p75<sup>NTR</sup>  
35

receptor.

12. The isolated nucleic acid molecule of claim 11,  
wherein the nucleic acid molecule encodes a  
5 polypeptide capable of binding p75<sup>NTR</sup> receptor set  
forth in Figure 1G-1 (SEQ ID NO: \_\_).
13. The isolated nucleic acid molecule of claim 3,  
wherein the nucleic acid molecule encodes a  
10 polypeptide capable of binding p75<sup>NTR</sup> receptor.
14. The isolated nucleic acid molecule of claim 9  
wherein the polypeptide capable of binding p75<sup>NTR</sup>  
receptor is mouse, rat or human protein.  
15
15. The isolated nucleic acid of claim 3 which  
comprises the nucleic acid sequence set forth in  
Figure 1G-1 (SEQ ID NO: \_\_).
- 20 16. A host cell comprising the vector comprising the  
nucleic acid molecule of claim 1.
17. The host cell of claim 16, wherein the cell is  
selected from a group consisting of a bacterial  
25 cell, a plant cell, an insect cell, and a  
mammalian cell.
18. A method of producing a polypeptide capable of  
binding p75<sup>NTR</sup> receptor which comprises growing  
30 the host cells of claim 17 under suitable  
conditions permitting production of the  
polypeptide.
19. The method of claim 18 further comprising  
35 recovering the produced polypeptide.
20. An isolated nucleic acid molecule of at least 15

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receptor.

- 5           30. A purified polypeptide capable of binding p75<sup>NTR</sup> receptor encoded by the isolated nucleic acid of claim 1.
- 10           31. A purified unique polypeptide fragment of the polypeptide capable of binding p75<sup>NTR</sup> receptor of claim 30.
- 15           32. The polypeptide capable of binding p75<sup>NTR</sup> receptor of claim 30 having substantially the same amino acid sequence as set forth in Figure 1G-1 (SEQ ID NO: \_\_).
- 20           33. The polypeptide capable of binding p75<sup>NTR</sup> receptor of claim 30 having the amino acid sequence as set forth in Figure 1G-1 (SEQ ID NO: \_\_).
- 25           34. The polypeptide capable of binding p75<sup>NTR</sup> receptor of claim 33 which is a vertebrate polypeptide capable of binding p75<sup>NTR</sup> receptor.
- 30           35. The polypeptide of claims 29-34 which comprises a neurotrophin associated cell death executor protein.
- 35           36. The polypeptide of claims 29-34 which comprises an amino acid sequence of NCLRILMGELSN.
37. The polypeptide of claim 35 which comprises an amino acid sequence of NCLRILMGELSN.
38. The vertebrate polypeptide capable of binding p75<sup>NTR</sup> receptor of claim 34 which is a mouse, rat, or human polypeptide capable of binding p75<sup>NTR</sup> receptor.



39. A monoclonal antibody directed to an epitope of a polypeptide capable of binding p75<sup>NTR</sup> receptor of claim 35.
- 5
40. A monoclonal antibody of claim 33 directed to a mouse, rat or human polypeptide capable of binding p75<sup>NTR</sup> receptor.
- 10
41. A polyclonal antibody directed to an epitope of the polypeptide capable of binding p75<sup>NTR</sup> receptor of claim 32.
- 15
42. A polyclonal antibody of claim 41 directed to a mouse, rat or human polypeptide capable of binding p75<sup>NTR</sup> receptor.
- 20
43. A method of inducing apoptosis in cells which comprises expressing a polypeptide capable of binding p75<sup>NTR</sup> receptor in the cells.
- 25
44. A method of inducing apoptosis in a subject which comprises expressing a polypeptide capable of binding p75<sup>NTR</sup> receptor in a subject.
- 30
45. The method of claim 44 where the subject is a rat, mouse or human.
- 35
46. A transgenic nonhuman mammal which comprises an isolated DNA molecule of claim 2.
47. The transgenic nonhuman mammal of claim 46, wherein the DNA encoding a polypeptide capable of binding p75<sup>NTR</sup> receptor is operatively linked to tissue specific regulatory elements.
48. A method of determining physiological effects of

expressing varying levels of a polypeptide capable of binding p75<sup>NTR</sup> receptor in a transgenic nonhuman mammal which comprises producing a panel of transgenic non human mammal expressing a different amount of polypeptide capable of binding p75<sup>NTR</sup> receptor.

49. A method of producing a polypeptide capable of binding p75<sup>NTR</sup> receptor into a suitable vector which comprises:

- (a) inserting a nucleic acid molecule encoding the polypeptide capable of binding p75<sup>NTR</sup> receptor into a suitable vector;
- (b) introducing the resulting vector into a suitable host cell;
- (c) selecting the introduced host cell for the expression of the polypeptide capable of binding p75<sup>NTR</sup> receptor;
- (d) culturing the selected cell to produce the polypeptide capable of binding p75<sup>NTR</sup> receptor; and
- (e) recovering the polypeptide capable of binding p75<sup>NTR</sup> receptor produced.

50. A method of inducing apoptosis of cells in a subject comprising administering to the subject a purified polypeptide capable of binding p75<sup>NTR</sup> receptor in an amount effective to induce apoptosis.

51. The method of claim 50 wherein the subject is a mammal.

52. The method of claim 51, wherein the mammal is mouse, rat or human.

53. A pharmaceutical composition comprising a purified polypeptide capable of binding p75<sup>NTR</sup> receptor of either claim 32 or 33 and a pharmaceutically acceptable carrier.

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54. A pharmaceutical composition comprising an effective amount of a purified polypeptide capable of binding p75<sup>NTR</sup> receptor of either claim 32 or 33 and a pharmaceutically acceptable carrier.

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55. A method of identifying a compound capable of inhibiting binding between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor comprising:

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a) contacting the compound with the polypeptide capable of binding to p75<sup>NTR</sup> receptor under conditions permitting the binding of the polypeptide capable of binding to p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> receptor to form a complex;

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b) contacting the p75<sup>NTR</sup> receptor with the mixture from step a); and

25

c) measuring the amount of the formed complexes or the unbound p75<sup>NTR</sup> receptor or the unbound polypeptide or any combination thereof.

56. A method of identifying a compound capable of inhibiting binding between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor, where said binding forms a complex between p75<sup>NTR</sup> receptor and a polypeptide capable of binding p75<sup>NTR</sup> receptor, comprising:

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a) contacting the compound with the p75<sup>NTR</sup> receptor under conditions permitting the

binding of the polypeptide capable of binding to p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> receptor to form a complex;

- 5           b) contacting the p75<sup>NTR</sup> receptor with the mixture from step a); and
- c) measuring the amount of the formed complexes or the unbound p75<sup>NTR</sup> receptor or the unbound polypeptide or any combination thereof.

10       57. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a neurotrophin associated cell death executor.

15       58. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a human HGR74 protein.

20       59. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a musnade3a sequence as defined on Figure 1H.

25       60. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a hunade3a1 sequence as defined on Figure 1H.

      61. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a hunade3a2 sequence as defined on Figure 1H.

30       62. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a ratnad3a sequence as defined on Figure 1H.

35       63. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a ratnad3b sequence as defined on Figure 1H.

64. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a musnade3b sequence as defined on Figure 1H.
- 5 65. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a humnade1 sequence as defined on Figure 1H.
- 10 66. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a ratnade1 sequence as defined on Figure 1H.
- 15 67. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a musnade1 sequence as defined on Figure 1H.
- 20 68. The method of claims 55 or 56 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a humnade2 sequence as defined on Figure 1H.
- 25 69. A method for identifying an apoptosis inducing compound comprising:
- 30 a) contacting a subject with an appropriate amount of the compound; and
- b) measuring the expression level of a polypeptide capable of binding p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene in the subject, an increase of the expression levels of a polypeptide capable of binding p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound.
- 35 70. The method of claim 69 wherein the subject is a mammal.

71. The method of claim 70, wherein the mammal is mouse, rat or human.

72. A method for identifying an apoptosis inducing compound comprising:

a) contacting a cell with an appropriate amount of the compound; and

b) measuring the expression level of a polypeptide capable of binding a p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene in the cell, an increase of the expression levels of polypeptide capable of binding p75<sup>NTR</sup> receptor gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound.

73. A method for screening cDNA libraries of a polypeptide capable of binding p75<sup>NTR</sup> receptor using a yeast two-hybrid system using a p75<sup>NTR</sup> intracellular domain as a target.

74. The method of claim 73 where the cDNA library is mammalian.

75. The method of claim 74 where the mammalian cDNA library is derived from rat, mouse or human cDNA libraries.

76. The method of claim 73 where the p75<sup>NTR</sup> intracellular domain target is mammalian.

77. The method of claim 76 where the mammalian p75<sup>NTR</sup> intracellular domain target is a rat, mouse or human p75<sup>NTR</sup> intracellular domain target.

78. A method to induce caspase-2 and caspase-3

activity to cleave poly (ADP-ribose) polymerase and fragment nuclear DNA in a cell by co-expression of polypeptide capable of binding p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>.

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79. A method to inhibit NF-κB activation in a cell with polypeptide capable of binding p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>.

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80. A method to detect a neurodegenerative disease in a subject by detecting expression levels of a polypeptide capable of binding p75<sup>NTR</sup> receptor and p75<sup>NTR</sup>.

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81. The method of claim 80 wherein the subject is a mammal.

82. The method of claim 81, wherein the mammal is mouse, rat or human.

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83. A transgenic nonhuman mammal which comprises an isolated nucleic acid, encoding a human HGR74 protein, which is a DNA molecule.

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84. The transgenic nonhuman mammal of claim 83 where the DNA encoding a human HGR74 protein is operatively linked to tissue specific regulatory elements.

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85. A method of determining physiological effects of expressing varying levels of human HGR74 in a transgenic nonhuman mammal which comprises producing a panel of transgenic non human mammal expressing a different amount of human HGR74 protein.

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86. A method of producing human HGR74 protein into a

suitable vector which comprises:

- 5 (a) inserting a nucleic acid molecule encoding a human HGR74 protein into a suitable vector;
  - (b) introducing the resulting vector into a suitable host cell;
  - (c) selecting the introduced host cell for the expression of the human HGR74 protein;
  - 10 (d) culturing the selected cell to produce the human HGR74 protein; and
  - (e) recovering the human HGR74 protein produced.
- 15 87. A method of inducing apoptosis of cells in a subject comprising administering to the subject the purified human HGR74 protein in an amount effective to induce apoptosis.
- 20 88. The method of claim 86 wherein the subject is a mammal.
89. The method of claim 87, wherein the mammal is mouse, rat or human.
- 25 90. A pharmaceutical composition comprising a purified human HGR74 protein and a pharmaceutically acceptable carrier.
- 30 91. A method for identifying an apoptosis inducing compound comprising:
- (a) contacting a subject with an appropriate amount of the compound; and
  - 35 (b) measuring the expression level of human HGR74 protein gene and p75<sup>NTR</sup> gene in the subject, an increase of the expression levels of human HGR74 protein and p75<sup>NTR</sup> gene



indicating that the compound is an apoptosis inducing compound.

- 5           92. The method of claim 91 wherein the subject is a mammal.
93. The method of claim 92, wherein the mammal is mouse, rat or human.
- 10       94. A method for identifying an apoptosis inducing compound comprising:
- (a) contacting a cell with an appropriate amount of the compound; and
- 15       (b) measuring the expression level of human HGR74 protein gene and p75<sup>NTR</sup> gene in the cell, an increase of the expression levels of human HGR74 protein gene and p75<sup>NTR</sup> gene indicating that the compound is an apoptosis inducing compound.
- 20           95. A method for screening cDNA libraries human HGR74 protein using a yeast two-hybrid system using a p75<sup>NTR</sup> intracellular domain as a target.
- 25           96. The method of claim 95 where the cDNA library is mammalian.
97. The method of claim 96 where the mammalian cDNA library is derived from rat, mouse or human cDNA libraries.
- 30           98. The method of claim 95 where the p75<sup>NTR</sup> intracellular domain target is mammalian.
- 35           99. The method of claim 98 where the mammalian p75<sup>NTR</sup> intracellular domain target is a rat, mouse or

human p75<sup>NTR</sup> intracellular domain target.

- 5           100. A method to induce caspase-2 and caspase-3 activity to cleave poly (ADP-ribose) polymerase and fragment nuclear DNA in a cell by co-expression of human HGR74 protein and p75<sup>NTR</sup>.
- 10           101. A method to inhibit NF-κB activation in a cell with human HGR74 protein and p75<sup>NTR</sup>.
102. A method to detect a neurodegenerative disease in a subject by detecting expression levels of human HGR74 protein and p75<sup>NTR</sup>.
- 15           103. The method of claim 102 wherein the subject is a mammal.
104. The method of claim 103, wherein the mammal is human.
- 20           105. A method of identifying a compound, which is an apoptosis inhibitor, said compound is capable of inhibiting specific binding between polypeptide capable of binding p75<sup>NTR</sup> receptor and p75<sup>NTR</sup> receptor, so as to prevent apoptosis which comprises:
- 25                           (a) contacting the polypeptide capable of binding p75<sup>NTR</sup> receptor with a plurality of compounds under conditions permitting binding between a known compound previously shown to be able to displace a polypeptide capable of binding p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor and the bound p75<sup>NTR</sup> receptor to form a complex; and
- 30                           (b) detecting the displaced polypeptide
- 35

capable of binding p75<sup>NTR</sup> receptor or the complex formed in step (a), wherein the displacement indicates that the compound is capable of inhibiting specific binding between the polypeptide capable of binding p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor.

106. The method of claim 105, wherein the inhibition of specific binding between the polypeptide capable of binding p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor affects the transcription activity of a reporter gene.

107. The method of claim 106, where in step (b) the displaced polypeptide capable of binding p75<sup>NTR</sup> receptor or the complex is detected by comparing the transcription activity of a reporter gene before and after the contacting with the compound in step (a), where a change of the activity indicates that the specific binding between the polypeptide capable of binding p75<sup>NTR</sup> receptor and the p75<sup>NTR</sup> receptor is inhibited and the polypeptide capable of binding p75<sup>NTR</sup> receptor is displaced.

108. The method of claim 105, wherein the p75<sup>NTR</sup> receptor is bound to a solid support.

109. The method of claim 105, wherein the compound is bound to a solid support.

110. The method of claim 105, wherein the compound comprises an antibody, an inorganic compound, an organic compound, a peptide, a peptidomimetic compound, a polypeptide or a protein.

111. The method of claim 105 wherein the contacting of step (a) is in vitro.
- 5 112. The method of claim 105, wherein the contacting of step (a) is in vivo.
113. The method of claim 112, wherein the contacting of step (a) is in a yeast cell.
- 10 114. The method of claim 112, wherein the contacting or step (a) is in a mammalian cell.
115. The method of claim 105, wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a cell surface receptor.
- 15 116. The method of claim 112, wherein the cell-surface receptor is the p75 receptor.
- 20 117. The method of claim 105 where in the polypeptide capable of binding p75<sup>NTR</sup> receptor is a neurotrophin associated cell death exectuor.
- 25 118. The method of claim 105 where in the polypeptide capable of binding p75<sup>NTR</sup> receptor is a human HGR74 protein.
- 30 119. The method of claim 105 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a neurotrophin associated cell death executor.
120. The method of claim 105 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a human HGR74 protein.
- 35 121. The method of claim 105 wherein the polypeptide capable of binding p75<sup>NTR</sup> receptor is a musnade3a

sequence as defined on Figure 1H.

122. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a hunade3a1  
5 sequence as defined on Figure 1H.

123. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a hunade3a2  
10 sequence as defined on Figure 1H.

124. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a ratnad3a  
sequence as defined on Figure 1H.

125. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a ratnad3b  
15 sequence as defined on Figure 1H.

126. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a musnade3b  
20 sequence as defined on Figure 1H.

127. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a humnade1  
25 sequence as defined on Figure 1H.

128. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a ratnade1  
sequence as defined on Figure 1H.

129. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a musnade1  
30 sequence as defined on Figure 1H.

130. The method of claim 105 wherein the polypeptide  
capable of binding p75<sup>NTR</sup> receptor is a humnade2  
35 sequence as defined on Figure 1H.

GENE ENCODING NADE, P75<sup>NTR</sup>-ASSOCIATED CELL DEATH  
EXECUTOR AND USES THEREOF

5     **Abstract of the Disclosure**

10     This invention provides an isolated nucleic molecule  
encoding a polypeptide capable of binding a p75<sup>NTR</sup>  
receptor, and a purified version of said polypeptide  
capable of binding a p75<sup>NTR</sup> receptor. This invention  
15     provides a method of producing a purified polypeptide  
capable of binding a p75<sup>NTR</sup> receptor. This invention  
provides an antisense oligonucleotide having a nucleic  
acid sequence capable of specifically hybridizing to  
an mRNA molecule encoding the above described  
20     polypeptide. This invention provides a method  
producing a polypeptide capable of binding p75<sup>NT</sup>  
receptor into a suitable vector. This invention  
provides a method of inducing apoptosis, a method of  
determining physiological effects, a method for  
25     identifying an apoptosis inducing or inhibiting  
compound, a method for screening cDNA libraries of  
said polypeptide, a method to induce caspase-2 and  
caspase-3 activity to cleave poly (ADP-ribose)  
polymerase and fragment nuclear DNA in a cell, a  
30     method to inhibit NF-κB activation in a cell, a method  
to detect a neurodegenerative disease, a method of  
producing the isolated human HGR74 protein into a  
suitable vector, a pharmaceutical composition  
comprising a purified polypeptide capable of binding  
a p75<sup>NTR</sup> receptor and a pharmaceutically acceptable  
35     carrier and a method of identifying a compound which  
is an apoptosis inhibitor.

\*\*\*\*\*  
\*\*\*\*\*

# Figure 1A

cZyxin	319-331	<u>L</u>	T	M	K	E	V	E	E	L	E	L	L	T
MAPKK	32- 44	A	<u>L</u>	Q	K	K	L	E	E	L	E	L	D	E
PKI- $\alpha$	37- 46		<u>L</u>	A	<u>L</u>	K	L	A	G	L	D	I		
TFIII A	330-338			<u>L</u>	P	V	L	E	N	L	T	L		
RevHIV-1	73- 81			<u>L</u>	P	P	L	E	R	L	T	L		
RanBP1	178-189	K	<u>V</u>	A	E	K	L	E	A	L	S	V	R	
FMRP	425-437	E	V	D	Q	<u>L</u>	R	L	E	R	L	Q	I	D
Gle1	351-356				<u>L</u>	P	L	G	K	L	T	L		
RexHTLV-1	81- 94	A	<u>L</u>	S	A	Q	L	Y	S	S	L	S	L	D S
human NADE	65- 77	R	E	<u>I</u>	R	R	K	L	R	E	L	Q	L	R
mouse NADE	88-100	R	E	<u>I</u>	R	R	K	L	R	E	L	Q	L	R

Figure 1 B



		Box 1										Box 2																
Mouse	88-114	R	E	I	R	R	K	L	R	E	L	Q	L	R	N	C	L	R	I	L	M	G	E	L	S	N	H	H
Human	75-101	R	E	I	R	R	K	L	R	E	L	Q	L	R	N	C	L	R	I	L	M	G	E	L	S	N	H	H
Consensus		R	X	X	L	X	X	L	X	-	-	N					R	X	X	L	X	X	L	X	N			

Figure 1 C

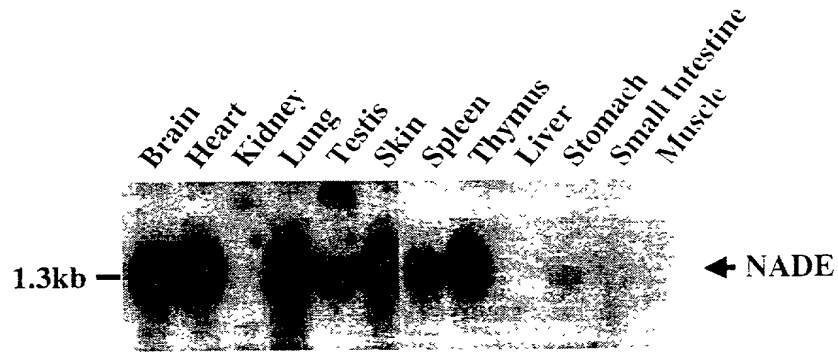


Figure 1 D

Figure 1E

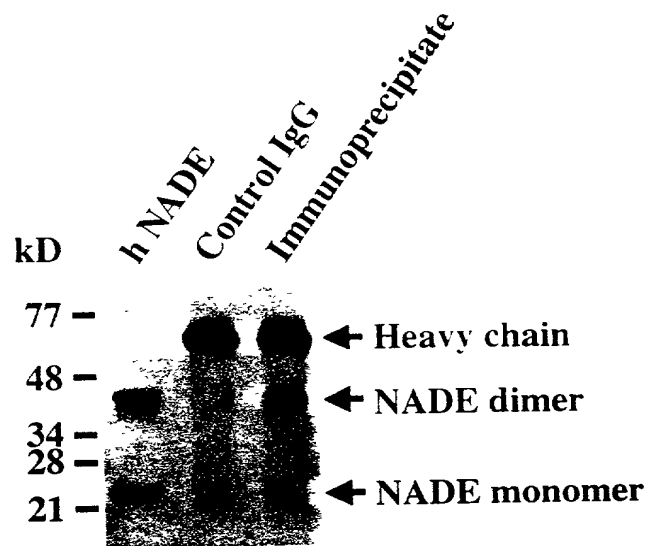


Figure 1F

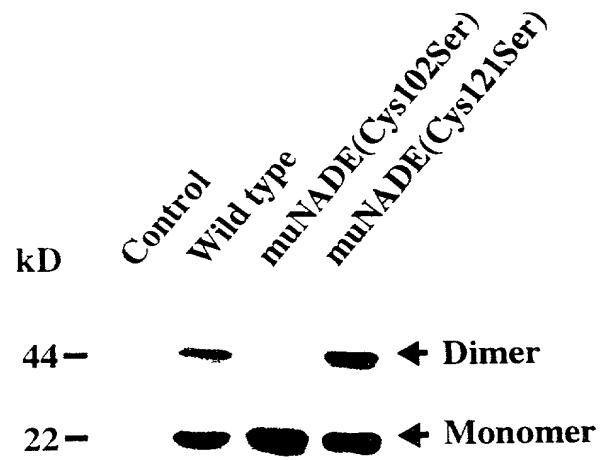


Figure 1G-1

**Mouse**

1 acgagcgtctggccagcagctcggagctcctctgcgcgcgggcggtggcagcgggcccg 60  
 61 aggcgagcgggacagattgactggaagccgagagtcaggcggcagcgggaattgacagg 120  
 121 aggactacgccgcaagggataggcccagaatagcaaccaggaaacaaaatctcatcatgg 180  
 181 ccaatgtccaccaggaaaacgaagagctggagcagccctgcagaatggacaggaagacc 240  
 241 gccctgtgggaggaggtgagggccaccagcctgctgcaaacaacaacaacaaccaca 300  
 301 accataaccacaaccaccaccgaagaggccaggctcgccgacttgccctaacttccgat 360  
 361 gggccattcccaacaggcagatgaatgacgggttgggtggagatggagatgatatggaaa 420  
 421 tgttcattggaggagatgagagagatccggagaaagcttagggagctacagctgagaaatt 480  
 481 gtctacgcctccttatgggggagctgtcttaaccaccacgataccatgatgaattctgcc 540  
 541 ttatgccttgacttcgggtcattccccccctgagatccatactgtgactcccgctgtagccc 600  
 601 ttttccctcgcattttccctgacatgcctttaatgaccgcgttctgtggtgagccttgtgttat 660  
 661 tcccatgccatgtgccagggtggggccttgtgttgccagtga

**Human**

1 accccatccccactcctataccggctcctccattttgggtgcctgcaaagctctgggaaaag 60  
 60 aatccccgggaaacgaaaaatgggtgggtttgggggaaggaggtaaggggagaaagctgga 120  
 121 gggaggggctttaattggaggccccgtagaggacgcgcggaacttctaagggtgggaaaaa 180  
 181 acgaaattaaaaaatcctttgatatacagggtctctgaatcctgctgggtcagagcaccaagc 240  
 241 attcagttctctctccttgcccttcttacttgtgttcaaagaaaaacaaccagaaaaaa 300  
 301 aaaatctcatcatggcaaatattcaccaggaaaacgaagagatggagcagcctatgcaga 360  
 361 atggagaggaagaccgcccccttggggaggaggtgaaggccaccagcctgcaggaaatcgac 420  
 421 ggggacaggctcgccgacttgccccataatttctgatgggccatacccaataggcagatca 480  
 481 atgatgggatgggtggagatggagatgatatggaaatattcatggaggagatgagagaaa 540  
 541 tcagaagaaaacttagggagctgcagttgaggaattgtctgcgtatccttatgggggagc 600  
 601 tctctaataccatgaccatcatgatgaatttgccttatgccttgactcctgccattta 660  
 661 tcatgagattaataactgtgattcccgtgttttcttttcttctgcatttcttaatatgc 720  
 721 ctttactgatccgtttgtgtgaacctatgttatttccatgtgtcaagtgggtcttctgtg 780  
 781 ttgccagcttctatttgaagattgcctttgcactcagtgtaagtttctgtcagcagtagt 840  
 841 ttcacccatttgcattggaaaaatttaaagctaataaagcaatttaaaaagc

Figure 1G-2

1	musnade3a	MESKD-QGVKNLAME	NDHQKKEKEKP-QDTIRREPAVALISEAG	KNCAPR	60	61	75	76	90
2	musnade3a	MESKEERALNNLIVE	NVNQENDEKDEKE-QVANKGEPL-ALPLANS	EYCVPR	60	61	75	76	90
3	musnade3a	MESKEKRAVNSLSME	NANQEN---EKE-QVANKGEPL-ALPLDAG	EYCVPR	60	61	75	76	90
4	musnade3a	MESKD-QGAKNLAME	NDHQKKEKEKP-QDTIKRPVVAFTFEAG	KNCAPR	60	61	75	76	90
5	musnade3a	MASKVKQVILDLTVE	KDKKKNKGGKASK-QSEES---HLLVEVEN	KKP	60	61	75	76	90
6	musnade3a	MASKVKQVILDLTVE	KDKKDKKGGKASK-QSEEP---HLLVEVEN	KKP	60	61	75	76	90
7	musnade1	MA	NIHQENEMEQQPM-QNGEEDRPLGGEGHQPA	MEQPL-QNGQEDRPFVGGEGHQPA	60	61	75	76	90
8	musnade1	MA	NVHQENEMEQQPL-QNGQEDRPFVGGEGHQPA	MEQPL-QNGQEDRPFVGGEGHQPA	60	61	75	76	90
9	musnade1	MA	NVHQENEMEQQPL-QNGQEDRPFVGGEGHQPA	MEQPL-QNGQEDRPFVGGEGHQPA	60	61	75	76	90
10	musnade2	ME	NVPKENKVVVEKAPVQN--EAPALGGGEYQEP	MEQPL-QNGQEDRPFVGGEGHQPA	60	61	75	76	90

1	musnade3a	GDVRQIME---KLRE	QQLSHSLRAVSTDDPP-	HHDDHDEFCLMP	130	135	136	150	151	165	166	180
2	musnade3a	BEVRQIME---KLRE	QQLSHSLRAVSTDDPP	-HHDDHDEFCLMP	130	135	136	150	151	165	166	180
3	musnade3a	BEVRQIME---KLRE	QQLSHSLRAVSTDDPP	-HHDDHDEFCLMP	125	135	136	150	151	165	166	180
4	musnade3a	EDMRQIME---KLRE	QQLSHSLRAVSTDDPP	-HHDDHDEFCLMP	125	135	136	150	151	165	166	180
5	musnade3a	RFVQGVMEAKRSKE	QQMRPYTRFTPEPD	NHYD---FCLIP	97	135	136	150	151	165	166	180
6	musnade3a	RFVQGVMEAKRSKE	QQMRPYTRFTPEPD	NHYD---FCLIP	97	135	136	150	151	165	166	180
7	musnade1	IFMEEMRETRKRLRE	LQLRNCILRLMGELS	NHDDHDEFCLMP	97	135	136	150	151	165	166	180
8	musnade1	IFMEEMRETRKRLRE	LQLRNCILRLMGELS	NHDDHDEFCLMP	97	135	136	150	151	165	166	180
9	musnade1	IFMEEMRETRKRLRE	LQLRNCILRLMGELS	NHDDHDEFCLMP	97	135	136	150	151	165	166	180
10	musnade2	RFMEEMRELRRKIRE	LQLRYSILRLIGDPP	-HHDDHDEFCLMP	97	135	136	150	151	165	166	180

Page 2.1

Figure 1H

Figure 2A

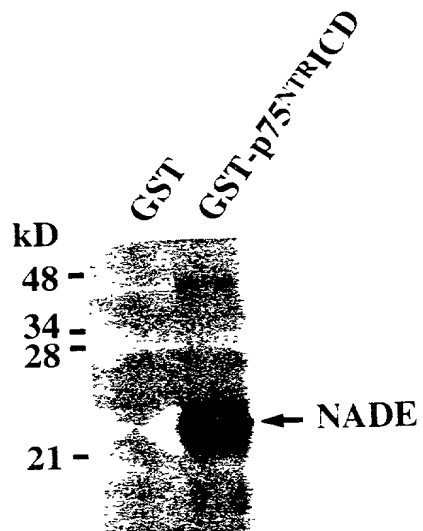
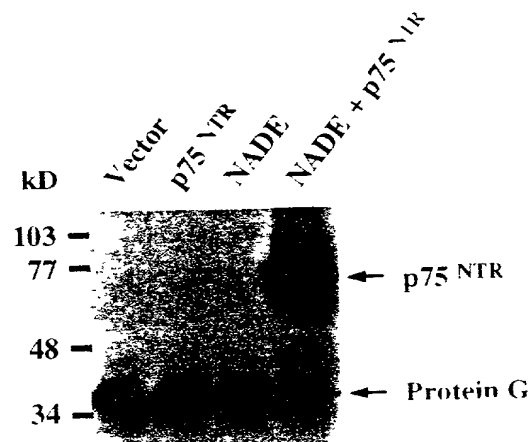
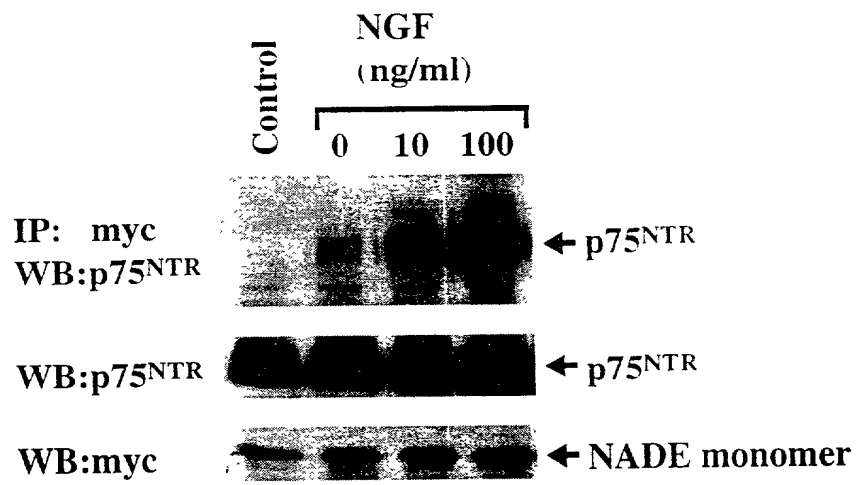
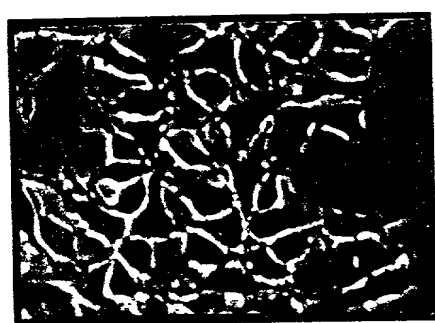


Figure 2B



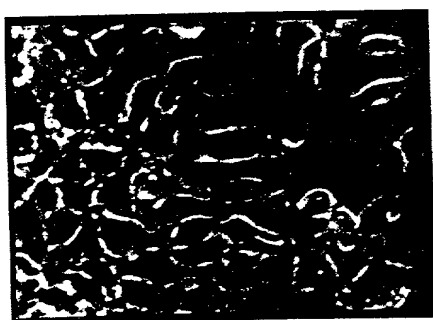
**Figure 2C**



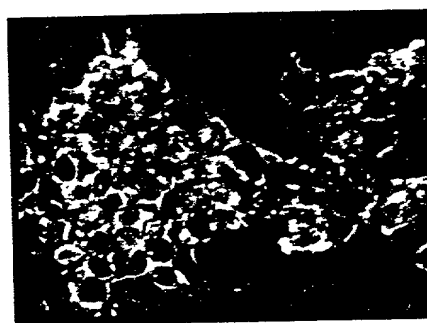
Control



NADE



p75<sup>NTR</sup>

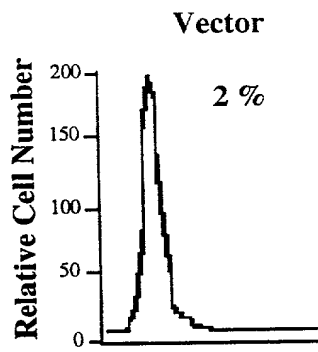


NADE + p75<sup>NTR</sup>

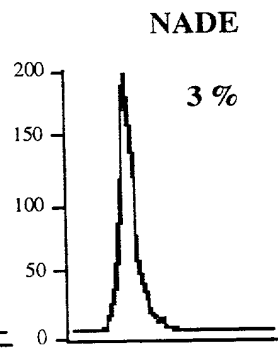
Figure 3 A



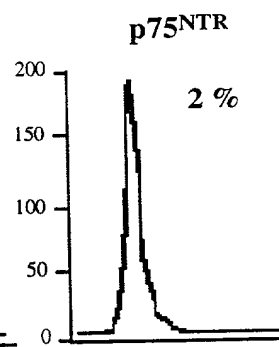
**Figure 3B-1**



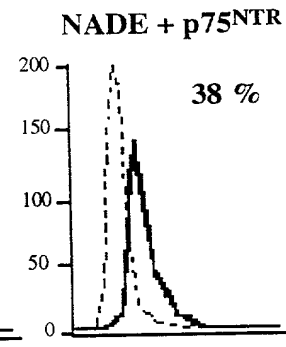
**Figure 3B-2**



**Figure 3B-3**



**Figure 3B-4**



Log (Fluorescence)

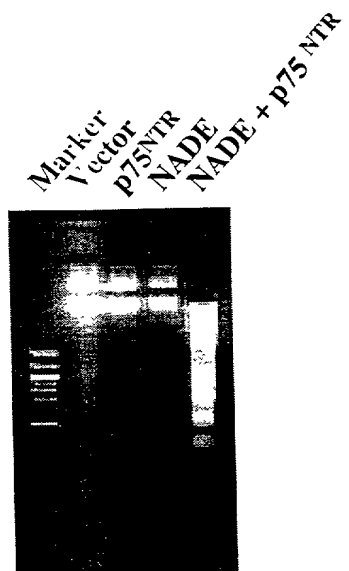


Figure 3C

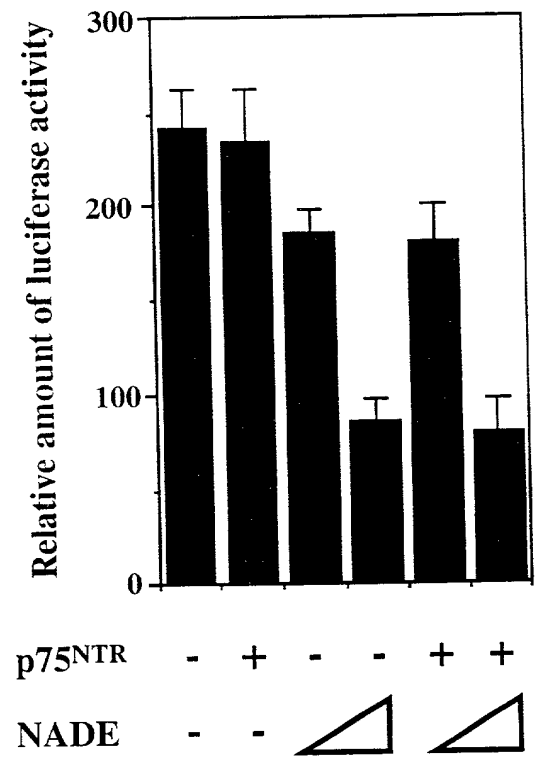


Figure 3D

14/16

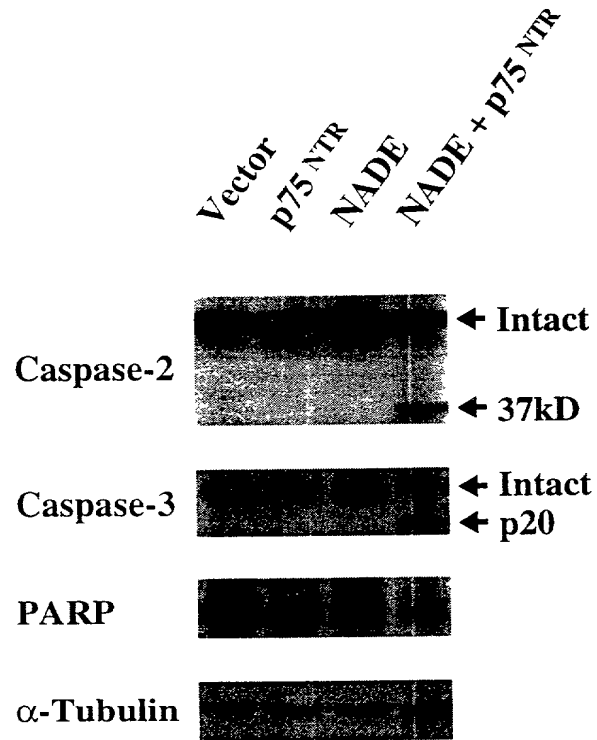
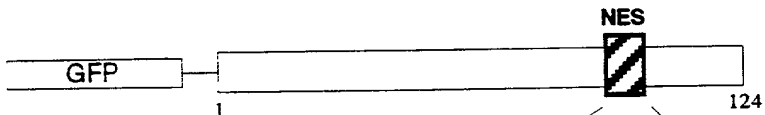


Figure 3E

### Figure 4A

**WT mouse NADE-GFP**



NADE NESs: mouse 88-100  
human 65-77

Other NESs: HIV rev 73-82  
MAPKK 32-43  
cZyxin 319-330  
PKI- $\alpha$  37-46

90 94 97 99  
REIRRKLRRLQLR  
REIRRKLRRLQLR  
LPPLERLRLTD  
ALQKKLEELRLD  
LTMKEVEELRL  
LALKLAGLDT

